Biomarkers of response and resistance to PI3K inhibitors in estrogen receptor-positive breast cancer patients and combination therapies involving PI3K inhibitors

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In this review, we discuss biomarkers of response and resistance to PI3K inhibitors (PI3Ki) in estrogen receptor-positive breast cancer, both in the early and advanced settings. We analyse data regarding PIK3CA mutations, PI3K pathway activation, PTEN expression loss, Akt signalling, insulin levels, ¹⁸FDG-PET/CT imaging, FGFR1/2 amplification, KRAS and TP53 mutations. Most of the discussed data comprise retrospective and exploratory studies, hence many results are not conclusive. Therefore, among all of these biomarkers, only PIK3CA mutations have proved to have a predictive value for treatment with the α-selective PI3Ki alpelisib (SOLAR-1 trial) and the β-sparing PI3Ki taselisib (SANDPIPER trial) in the advanced setting. Since the accuracy of current individual biomarkers is not optimal, a composite biomarker, including DNA, RNA and protein expression data, to more precisely assess the PI3K/AKT/mTOR pathway activation status, may arise as a promising approach. Finally, we describe the rational for new combination therapies involving PI3Ki and anti-HER2 agents, chemotherapy, CDK4/6 inhibitors, mTOR inhibitors or new endocrine treatments and discuss the ongoing trials in this field.

Key words: breast neoplasms, predictive biomarkers, PI3K inhibitors, PIK3CA, gene sequencing

Introduction

Since the landmark BOLERO-2 trial demonstrated the benefit of targeting the PI3K/AKT/mTOR pathway in breast cancer (BC) [1], there has been an enormous effort to find new agents and innovative combinations targeting this pathway. Yet, given the toxicities and costs associated with these agents, research has focused on ways to better identify which patients would benefit the most from these treatments. In this review, we will discuss biomarkers of response and resistance specifically to PI3Ki in estrogen receptor (ER)-positive BC. Furthermore, we will describe the rationale for new combination therapies involving PI3Ki and the ongoing trials evaluating these strategies.

Potential biomarkers to predict the benefit from PI3Ki

PIK3CA gene mutations and PI3K pathway activation status

Preclinical studies show that PIK3CA-mutated (PIK3CA-mut) BC cells are more sensitive to PI3Ki [3, 4], yet clinical data assessing its predictive value are contradictory (Table 1). In the
A potential reason for PIK3CA mutational status contradictory results may be due to its variable oncogenic potential, leading to different degrees of tumour cells’ addiction to PI3K/AKT/mTOR pathway activation [24]. Indeed, Loi et al. have developed a PIK3CA-mutant-related gene signature (PIK3CA-GS), which could predict the occurrence of mutations in PIK3CA and AKT1, and was correlated with a PTEN-loss gene signature [25]. Interestingly, the authors showed that higher PIK3CA-GS scores (i.e. corresponding to the mutant-like phenotype) were associated with low levels of pathway activation. On the other hand, patients with lower PIK3CA-GS scores (i.e. with higher pathway activation) had the greater benefit from treatment with letrozole/everolimus [25, 26]. Moreover, Mertins et al. showed that some PIK3CA-mut breast tumours do not present downstream pathway activation, further demonstrating the variable oncogenic potential of the PIK3CA-mut [27]. One possibility is that the tumour may require another hit for full activation of the pathway and, indeed, it has recently been demonstrated that the occurrence of double PIK3CA mutations in cis leads to an increased PI3K pathway activity and downstream signalling in breast tumours compared with single hotspot mutations [28]. In addition, these double mutations rendered tumours more sensitive to α-selective PI3Ki.

On the other hand, even in PIK3CA-mut tumours, resistance to PI3Ki can be mediated by activation of alternative pathways that drive cell proliferation (MAPK, ER, HER2, AXL, PIM-1, FOXO transcription factors); by signalling via other PI3K isoforms when a specific subunit is blocked; by activation of downstream effectors in the PI3K pathway such as AKT and mTOR; by loss of regulators of PI3K signalling such as PTEN; or by epigenomic crosstalk between PI3K and ER pathways, resulting in upregulation of ER-dependent transcription upon PI3K inhibition (Figure 1) [2, 29–32]. In order to overcome this classification issue, studies in metastatic BC have also analysed the benefit from PI3Ki according to a ‘PI3K pathway activation’ status biomarker. Its definition, however, varied between studies and usually combined DNA with protein expression assessment (Table 1). Even so, none of these PI3Ki trials indicated a predictive value of an ‘activated PI3K pathway’ biomarker [7, 9].

The OPPORTUNE trial suggested that patients with progesterone-receptor-negative or luminal B tumours may benefit more from pictilisib due to the drug’s antiproliferative effect [5, 6], but this was not demonstrated in the NEO-ORB trial [18].

Other potential biomarkers of response

Other explanation to these contradictory results may relate to tumour heterogeneity—in some cases, PIK3CA mutations may not be early clonal events, but subclonal drivers present only in a part of the metastatic lesions and, thus, targeting this pathway may be less efficacious. Yet, results from the AURORA program show a high concordance rate of PIK3CA-mut between primary tumours and matched metastasis, suggesting that, in most cases, detected PIK3CA-mut are clonal [33]. On the other hand, its predictive value may change according to specific targeted agents—a hint to this differential effect is given by the β-sparing and α-selective PI3Ki studies [17, 22]: a predictive effect of PIK3CA-mut was demonstrated in both trials, but not in all trials testing pan-PI3Ki. PIK3CA mutations’ predictive value may also depend on disease setting: its oncogenic potential may be less important in the early when compared with the advanced setting [34], in which it has a role on the development of resistance to endocrine treatment [2]. Thus, its presence in endocrine treatment-resistant tumours in the advanced setting may predict benefit from targeted inhibition with PI3Ki, but not in ‘treatment-naïve’ tumours, like the ones in the neoadjuvant setting.

Potential biomarkers of resistance to PI3Ki

PTEN expression loss

Preclinical data suggest that cells with PTEN expression loss are more sensitive to AKT/PI3K inhibitors (PI3Ki) [4]. Juric et al. reported the case of a patient who progressed while being treated with alpelisib, in which all progressing metastatic lesions showed a de novo loss of PTEN expression, by different but convergent genetic alterations [36]. Then, the authors functionally analysed PTEN-null xenografts derived from this patient, which were also resistant to alpelisib. On the other hand, it is known that PTEN-deficient tumours are dependent on PI3Kβ signalling [37] and this may explain why patients included in the OPPORTUNE trial derived benefit from the pan-PI3Ki pictilisib (which also targets PI3Kβ), whether they had PTEN-positive or PTEN-negative tumours [5, 6]. Nonetheless, assessment of ‘PTEN status’ can be challenging, as its ‘loss’ has been determined by the allelic or complete loss of the PTEN gene [19, 21], but also by the
| Trial               | Phase | Population | Treatment | Tested tissue | Mutated/altered population | WT/normal/ITT population | Comments/ conclusion |
|--------------------|-------|------------|-----------|---------------|-----------------------------|--------------------------|-----------------------|
| Pan-Pi3K           | II    | ER+/HER2–, operable BC (≥2 cm), n=167 → 75 initial analysis, 136 evaluable final analysis | Anastrozole+ pictilisib×2w | IHC (Ki67, PGr, PTEN)+ targeted NGS of >400 genes + CNV analysis + reverse-phase protein arrays and RNA profiling. BC subtype was defined using the NanoString PAM50 algorithm | PIK3CA-mut overall (n=49): geometric mean KI67 suppression ratio (at D15) 0.72 (95% CI 0.46–1.15) | PIK3CA-WT: geometric mean KI67 suppression ratio: 0.63 (95% CI 0.39–1.0) | No predictive value (overall), differences according to type of mutation? |
| Neoadjuvant        |       |            |           |               |                             |                          |                       |
| OPPORTUNE [5, 6]   |       |            |           |               |                             |                          |                       |
|                    |       |            |           |               |                             |                          |                       |
| Metastatic         |       |            |           |               |                             |                          |                       |
| BELLE-2 [7]        | III   | ER+/HER2–, after AI, n=1147 | Fulvestrant+ buparlisib | Archived primary tumour tissue— IHC (PTEN) and analysis of PIK3CA (exons 1, 7, 9, and 20) by Sanger sequencing, n=851 Blood (ctDNA) at baseline—analysis of PIK3CA (exons 1, 7, 9, and 20) by PCR, n=587 PI3K pathway activated [b,c] (44%); PFS HR 0.76 (95% CI 0.60–0.97); OS HR 0.81 (95% CI 0.61–1.08) | PIK3CA-mut: PFS HR 0.58 (95% CI 0.41–0.82); OS HR 0.81 (95% CI 0.56–1.17) | ITT (with known PI3K status): PFS HR 0.80 (95% CI 0.68–0.94); non-activated PI3K pathway: OS HR 0.98 (95% CI 0.77–1.24) | Predictive value: benefit only in PIK3CA-mut |
| BELLE-3 [8]        | III   | ER+/HER2–, after ET+ everolimus, n=432 | Fulvestrant+ buparlisib | New or archived (73%) tissue— analysis of PIK3CA (exons 7, 9 and 20) by PCR, n=320 | PIK3CA-mut: PFS HR 0.53 (95% CI 0.33–0.83) | PIK3CA-WT: PFS HR 0.81 (95% CI 0.59–1.12) | Predictive value: benefit only in PIK3CA-mut |
| BELLE-4 [9]        | II/III| HER2−, no prior CT for ABC, prior ET allowed; n=416 302 ER+ [73%] | Paclitaxel+ buparlisib | Archived (most) or fresh biopsy tissue— IHC (PTEN) and analysis of PIK3CA (exons 1, 7, 9, and 20) by Sanger sequencing | PIK3CA-mut: PFS HR 0.46 (95% CI 0.29–0.73) | PIK3CA-WT: PFS HR 0.73 (95% CI 0.53–1.00) | No predictive value |
| FERGI [10]         | II    | Part 1: ER+/HER2–, after AI; n=168 | Fulvestrant+ pictilisib 340 mg | Tissue (not specified)—analysis of PIK3CA missense mutations (C202R, E542K, E545A, E545G, or E545K, and H1047L, H1047R, or H1047Y) by PCR | PIK3CA-mut[b,c]: PFS HR 0.73 (95% CI 0.42–1.28) | PIK3CA-WT: PFS HR 0.72 (95% CI 0.42–1.23) | No predictive value |
|                     |       | Part 2: ER+/HER2–, after AI; only PIK3CA-mut, n=61 | Fulvestrant+ pictilisib 260 mg | | | | |
| PEGGY [11]         | II    | ER+/HER2–, first-/second-line CT for ABC, n=183 | Paclitaxel+ pictilisib 260 mg | Archived primary tumour or fresh biopsy metastatic tissue—analysis of PIK3CA (exons 7, 9, and 20) by PCR, n=168 | PIK3CA-mut[b,c]: PFS HR 0.73 (95% CI 0.42–1.28) | PIK3CA-WT: PFS HR 0.72 (95% CI 0.42–1.23) | No predictive value |
|                     |       |            |           |               |                             |                          |                       |

Continued
| Trial | Phase | Population | Treatment | Tested tissue | Mutated/altered population | WT/normal/ITT population | Comments/conclusion |
|-------|-------|------------|-----------|---------------|-----------------------------|--------------------------|---------------------|
| -Sparing PI3Ki | | | | | | | |
| NEO-ORB [18] | II | ER+HER2−, T1c-T3, n=257 | Letrozole ± alpelisib x24w | Primary BC—analysis of PIK3CA (exons 1, 4, 7, 9, and 20) by PCR | PIK3CA-mut: ORR 43% (alpelisib) versus 45% (P), P=0.435; pCR 1.7% (alpelisib) versus 3.0% (P), P=0.282 | PIK3CA WT: ORR 63% (alpelisib) versus 61% (P), P=0.611; pCR 28% (alpelisib) versus 17% (P), P=0.697 | No predictive value |
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| METASTATIC | | | | | | | |
| Saura [14] | I | ER+ ABC, after ≥1 ET line, n=28 | Letrozole + taselisib | Archived or fresh tissue—analysis of PIK3CA (exons 1, 4, 7, 9, and 20) by PCR | PIK3CA-mut: CBR 38.5% (95% CI 13.9–68.4) | NA | Numerically higher CBR in the PIK3CA-mut group |
| | | | | | | | |
| Dickler [15] | I | ER+HER2− ABC, after ≥1 ET line, n=47 | Fulvestrant + taselisib | Archived or fresh tumour biopsy and blood (ctDNA)—analysis of PIK3CA mutations (method?) | PIK3CA-mutf: ORR: 33%, CBR 58%, mPFS 7.9 months (95% CI 5.6–11.8) | NA | No predictive value |
| | | | | | | | |
| PIPA [16] | I | ER+HER2− PIK3CA-mutant ABC cohort, after ≥1 line of ET, n=24 | Fulvestrant + taselisib + palbociclib | Archival or fresh tumour biopsy and blood (ctDNA)—analysis of PIK3CA mutations (method?) | PIK3CA-mutg: ORR: 33%, CBR 58%, mPFS 7.9 months (95% CI 5.6–11.8) | NA | No predictive value |
| | | | | | | | |
| SANDPIPER [17] | II | Cohort PIK3CA-mut: ER+/HER2− ABC, after Al, n=516 | Fulvestrant + taselisib | Archived or fresh tumour analysis of PIK3CA (exons 1, 4, 7, 9, and 20) by PCR | PIK3CA-mut: ORR 33%, CBR 58%, mPFS 7.9 months (95% CI 5.6–11.8) | NA | Predictive value: benefit only in PIK3CA-mut (but similar HR) |

Continued
| Trial                        | Phase | Population                          | Treatment                  | Tested tissue                                                                 | Mutated/altered population                                                                 | WT/normal/ITT population | Comments/conclusion                                                                 |
|-----------------------------|-------|-------------------------------------|----------------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------------------|--------------------------------------------------------------------------------------|
| Juric [20]                  | I b   | ER+/HER2− ABC, endocrine-resistant (heavily pre-treated), n=81 | Fulvestrant + alpelisib | Archive or fresh tumour biopsy—analysis of PIKCA by NGS                      | PIK3CA-mut: ORR 29% (95% CI 17% to 43%), mPFS 9.1 months (95% CI 6.6–14.6)                 | NA                     | Numerically higher ORR in the PIK3CA-mut group                                         |
| Sharma [21]                 | I/II  | HER2− ABC, after ≥1 line of CT (any setting), n=43 (of which 70% were ER+) | Navel-paclitaxel + alpelisib | Tumour tissue and blood (ctDNA)—analysis of PIKCA and PTEN mutations by NGS | PIK3CA pathway activated (n=19, 44%); mPFS 13 months (95% CI 9–17)                          | NA                     | Apparent prognostic value (PFS HR 0.40; 95% CI 0.18–0.90)                              |
| SOLAR-1 [22, 23]            | III   | Cohort PIK3CA-mut ER+/HER2− ABC, after AI, n=341 | Fulvestrant ± alpelisib | Archived or fresh tissue—analysis of PIK3CA (exons 7, 9, and 20) by PCR;     | PIK3CA-mut in tissue: PFS HR 0.65 (95% CI 0.50–0.85)                                      | NA                     | Predictive value: benefit only in PIK3CA-mut, independent of exon or type of mutation|
|                             |       | Cohort PIK3CA WT. ER+/HER2− ABC, after AI, n=231 |                            | Blood (ctDNA) at baseline (secondary end point)—analysis of PIK3CA (exons not described) by PCR | PIK3CA-mut in ctDNA (n=186): PFS HR 0.55 (95% CI 0.39–0.79)                               | NA                     |                                                                                       |

*a* Definition of activated PI3K pathway: OPPORTUNE: not defined.

*b* Stratification factor and/or assignment criteria to a specific treatment cohort.

*c* Definition of activated PI3K pathway: BELLE-2 and BELLE-4: PI3K pathway activated: PIK3CA-mutation and/or no PTEN expression (by immunohistochemistry).

*d* Primary end point.

*e* Definition of activated PI3K pathway: Sharma et al.: PI3K pathway activated: presence of PIK3CA-activating or PTEN-inactivating mutations in either tumour tissue or ctDNA.

ABC, advanced breast cancer; AI, aromatase inhibitor; BC, breast cancer; CBR, clinical benefit rate; CI, confidence interval; CNV, copy number variations; CT, chemotherapy; ctDNA, circulating tumour DNA; ER+, estrogen receptor positive; ET, endocrine therapy; FISH, fluorescent in situ hybridization; HER2+, HER2 positive; HER2−, HER2 negative; HR, hazard ratio; IHC, immunohistochemistry; ITT, intention-to-treat population; mPFS, median progression-free survival; mut: mutation; NA, not applicable; NGS, next-generation sequencing; OR, odds ratio; ORR, overall response rate; P, placebo; PCR, polymerase chain reaction; PFS, progression-free survival; PgR, progesterone receptor; PIK3CA-mut, mutation in the PIK3CA gene; Ph, phase of the clinical trial; T, tumour size; WT, wild-type.
immunohistochemical expression of the PTEN protein or of other downstream markers, like phosphorylated-Akt [5, 7, 9, 12, 13, 38]. Moreover, these studies have used different PTEN antibodies and variable definitions of PTEN status, making comparisons difficult between them.

High insulin levels

It is well known that PI3K mediates cellular responses to insulin and that its inhibition leads to hyperglycaemia [17, 22]. A recent report has shown that PI3Ki-induced hyperglycaemia leads to an increase in insulin release and that this is sufficient to re-activate PI3K signalling in tumour models in mice, even in the presence of PI3Ki, leading ultimately to treatment resistance [39]. The authors have also demonstrated that this insulin feedback can be prevented or attenuated using dietary (e.g. ketogenic diet) and pharmacological measures (e.g. sodium-glucose cotransporter inhibitors), which improve the efficacy of PI3Ki. On the other hand, administration of exogenous insulin to control hyperglycaemia could further activate PI3K signalling in tumour cells and impair the efficacy of PI3Ki.

This hypothesis could partly explain why in the SANDPIPER trial there were differences in taselisib efficacy according to the region of the world: the hazard ratio (HR) was 0.38 [95% confidence interval (CI) 0.19–0.75] in Asia, 0.57 [95% CI 0.41–0.79] in Western Europe/USA/Canada/Australia, and 1.18 [95% CI 0.78–1.77] in other regions.

Figure 1. Mechanisms of resistance to PI3K inhibitors in estrogen receptor (ER)-positive breast cancer and current and future drug combination strategies involving PI3K inhibitors. In PIK3CA-mutated breast tumours, resistance to PI3K inhibitors can be mediated by multiple mechanisms, including activation of alternative pathways that drive cell proliferation (e.g. RAS/MEK/ERK pathway, ER pathway, or HER2 pathway); by signalling via other PI3K isoforms when a specific subunit is blocked; by activation of downstream effectors in the PI3K pathway such as Akt and mTOR; by loss of regulators of PI3K signalling such as PTEN; or by epigenetic crosstalk between PI3K and ER pathways, resulting in upregulation of ER-dependent transcription. Ab, monoclonal antibody; AR, androgen receptor; CDK4/6i, CDK4/6 inhibitors; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; IGFR1, insulin growth factor receptor 1; mTOR, mTOR inhibitors; PI3Ki, PI3K inhibitors; SERD, selective estrogen receptor degraders; T-DM1, ado-trastuzumab emtansine; TKI, tyrosine kinase inhibitor. Dashed arrows, inhibitory function; bold arrows, activation function. Note: within each drug class, we have only included compounds that have been or that are currently being tested in combination with PI3K inhibitors in clinical trials (see Tables 2 and 3 for more details).
Latin America/Eastern Europe [20]. Thus, differences in patients’ degree of insulin resistance, diet, and in management of hyperglycaemia (e.g. insulin use) according to each region could justify these discrepancies.

Although there is not yet clinical data to support this hypothesis, this is being explored on the datasets from PI3Ki clinical trials. Some PI3Ki trials already recommended the preferential use of oral antidiabetic drugs for hyperglycaemia management [12, 22]. Yet, if this hypothesis is proved, this would lead to further adoptions on the design of clinical trials using PI3Ki and also on the selection and follow-up of patients in daily clinical practice.

Other biomarkers of resistance

Data suggest that in PI3Ki-resistant cell lines there is low-level Akt signalling, and these can be resensitized by using the AKTi MK-2206 [32]. Nonetheless, a phase I trial combining neoadjuvant MK-2206 with anastrozole in patients with PIK3CA-mut tumours showed incomplete target inhibition and lack of further Ki67 suppression [40].

A small number of patients with FGFR1/2 amplification, KRA5 or TP53 mutations did not derive clinical benefit in a phase Ib trial of letrozole/alpelisib [19], but this needs confirmation in larger studies.

**Limitations of biomarker research study**

Despite intense research efforts to find predictive biomarkers of response/resistance to PI3Ki, so far only PIK3CA mutations (detected either in tissue or blood) have been approved by the US Food and Drug Administration (FDA) as a predictive biomarker for the use of alpelisib [41]. Moreover, PIK3CA mutations have been recently classified in the tier of evidence IA of genomic alterations in breast cancer (BC) of the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT), as predictors of benefit from α-selective PI3Ki [42].

There are several reasons that may explain the lack of definitive findings regarding the predictive value of PIK3CA-mut with other PI3Ki or for the other biomarkers studied so far. The first is that many of these analyses are retrospective, exploratory and based on a small number of patients. As some of the tested genetic alterations (e.g. mutations in PTEN) have a low frequency, statistical challenges and the risk of overfitting exist. Only the more recent trials have prospectively assessed PIK3CA-mut status or PI3K pathway activation before patients inclusion [7, 9, 10, 16–18, 22], but still the predictive value of PIK3CA-mut was only proven in the β-sparing and α-selective PI3Ki trials. This may partly be explained by the low tolerability of pan-PI3Ki when compared with isofom-selective PI3Ki: exposure to pan-PI3Ki was often reduced due to their toxicity, and this could have led to inadequate pathway inhibition. Thus, it may have confounded the interpretation of biomarker data, leading to contradictory results in the pan-PI3Ki trials [5–11].

Furthermore, differences in methods (use of Sanger sequencing, PCR, next-generation sequencing, etc.) and types of mutations assessed may have also influenced results. Lastly, these biomarkers have been mostly evaluated at baseline only. Data from CDK4/6i trials show that tumour genome may change under selective therapeutic pressure [43], thus it would be interesting to assess genetic alterations over time in patients treated with PI3Ki as well. A convenient technique to perform this would be through circulating tumour (ct)DNA [44]. Another issue relates to timing of assessment: most trials tested biomarkers on archived tissue, usually the primary breast tumour. Some of them assessed the concordance of PIK3CA-mut status between (archived) tissue versus ctDNA and it varied between 70% and 83% [7, 8, 45]. Interestingly, in BELLE-2, 21% patients with PIK3CA-wild-type tumour tissue had PIK3CA-mut ctDNA, which suggests tumour evolution between initial diagnosis and the time at which patients started a PI3Ki [7]. Thus, biomarkers like PIK3CA-mut should be assessed (either in blood or in a recent tissue biopsy) at the time of PI3Ki treatment initiation and not in archived tissue. Of note, in SOLAR-1, the number of patients with a PIK3CA-mut in ctDNA was lower than in the archival tissue (186 versus 341 patients, respectively), thus suggesting that a proportion of patients with PIK3CA-mut tissue had no identifiable PIK3CA-mut in ctDNA [23]. This is in line with the preliminary findings from the AURORA program, in which more than half of patients with a PIK3CA-mut identified in metastatic tissue (taken just before inclusion) did not present an identifiable PIK3CA-mut on synchronous ctDNA [33], which may be explained by many factors (e.g. low tumour burden, among others). This is the reason why FDA recommends that patients who have a negative ctDNA PIK3CA-mut test should undergo tumour biopsy for PIK3CA-mut assessment [41]. Still, as in SOLAR-1, patients with PIK3CA-wild-type ctDNA did not benefit from the addition of alpelisib (HR 0.80; 95% CI 0.60–1.06), it would be important to analyse the benefit of alpelisib in the subgroup of ‘discordant’ patients, who have PIK3CA-mut tissue, but a PIK3CA-wild-type ctDNA.

**Future research**

Genomic, transcriptomic and proteomic information assessed in breast tumour samples from clinical trials testing PI3Ki should be publicly available. This would allow the combination of all this information, in order to better understand the predictive and prognostic role of PIK3CA-mut and other genetic alterations in advanced BC. Furthermore, future trials should prospectively assess these biomarkers, not only at baseline but throughout treatment and at disease progression. As an example, there is an ongoing prospective trial (CICLADES, NCT03318263), longitudinally assessing ctDNA for ESR1, PIK3CA and AKT1 mutations during first-line endocrine treatment with/without targeted therapy, in order to assess their predictive value.

As immunotherapy is emerging as a possible treatment of BC patients, we should also assess the effects of the different PI3Ki on tumour microenvironment and how it can predict response to these treatments. Finally, as new combination therapies involving PI3Ki are being developed, biomarkers to predict which patients will benefit from them should also be sought.

**Combination therapies involving PI3Ki**

Antitumour activity of PI3Ki in preclinical studies is encouraging, and β-sparing and α-selective PI3Ki have demonstrated to be
effective in metastatic BC patients with PIK3CA-mut tumours [17, 22]. Nonetheless, disease progression invariably occurs during PI3Ki treatment, and therefore strategies to overcome resistance and improve patients’ outcomes are necessary. Given the resistance mechanisms previously described, combination of PI3Ki with targeted therapies that suppress alternative pathways, or blockade of PI3K at downstream levels are potential strategies to overcome resistance (Figure 1; Tables 2 and 3).

**With anti-HER2 agents**

In preclinical models of HER2-positive BC cells, PI3K pathway activation induces resistance, while treatment with PI3Ki restores sensitivity to anti-HER2 therapies, and the combination of anti-HER2 with PI3Ki has synergic antitumour activity [61]. Likewise, in HER2-positive BC patients, presence of a PIK3CA-mut is associated with worse response rates to neoadjuvant treatment [62]. Phase I/II studies demonstrated the overall feasibility of combining anti-HER2 treatments with PI3Ki (Table 2) [46, 50–59, 63]. A phase II study including HER2-positive, trastuzumab-resistant metastatic BC patients treated with buparlisib and trastuzumab showed an overall response rate of 10%, but grade ≥ 3 toxicities were observed in 70% of patients [52]. The NeoPHOEBE trial randomized HER2-positive BC patients to neoadjuvant trastuzumab/paclitaxel with/without buparlisib. This trial was interrupted after enrolment of only 50 patients due to an increased incidence of severe liver toxicity. Pathological complete response rates did not differ between buparlisib and placebo, yet a significant decrease in Ki67 was observed with buparlisib (75%) versus placebo (26.7%), suggesting that PI3Ki may be active in HER2-positive BC [63]. Despite this promising activity, the high frequency of severe toxicities was concerning. As isoform-selective PI3Ki might have a more favourable toxicity profile, ongoing studies are evaluating their combination with anti-HER2 treatments in HER2-positive BC patients (Table 3) [46].

**With chemotherapies**

PI3K pathway activation induces resistance to chemotherapy in BC cells [64]. In most clinical studies evaluating PIK3CA-mut as a predictor of chemotherapy response in BC, inferior response rates were observed in patients with PIK3CA-mut tumours when compared with patients with PIK3CA-wild-type tumours [62, 65]. Therefore, the combination of PI3Ki and chemotherapy is being investigated as an attempt to overcome treatment resistance, but no promising results have been observed so far (Table 2) [9, 11, 21, 49, 59]. Ongoing trials are evaluating the association of PI3Ki with chemotherapy in HER2-negative BC (Table 3).

**With CDK4/6 inhibitors**

Cyclin-dependent kinases (CDK) are involved in cell cycle regulation, and the dysregulated activation of these proteins is a mechanism of resistance to endocrine treatment [66]. Preclinical studies demonstrated an interaction between the CDK4/6 and PI3K pathways in ER-positive BC cells: the antitumour effect of CDK4/6 inhibitors (CDK4/6i) was impaired with PI3K/AKT/mTOR pathway activation, while in BC cells harbouring a PIK3CA-mut, co-treatment with CDK4/6i and PI3Ki was more effective than a PI3Ki alone. This suggested that PI3K activation is a potential mechanism of resistance to CDK4/6i [32]. Early phase trials have already shown the combination of CDK4/6i and PI3Ki may be active in BC (Table 2) [16, 47, 48]. To further explore their potential synergistic effect, ongoing studies are currently evaluating their combination (Table 3).

**With new endocrine agents**

Survival and proliferation of ER-positive BC cells is highly dependent on ER signalling [67]. ER pathway can remain active, even in the presence of endocrine treatment, through mutations in ESR1 gene, or via the activation of downstream effectors by alternative kinases such as PI3K, HER2 and MAPK [68]. The selective ER modulators/degraders (SERMs/SERDs) are agents designed to bind to the ER, block its signalling and/or increase its degradation. There are new SERDs/SERMs with the potential to bind to the mutated ER and thereby restore the effective blockade of the ER pathway in ESR1-mutated BC cells. Since ESR1-mutation and PIK3CA-mut are both involved in endocrine resistance in ER-positive BC, an ongoing study is evaluating the combination of a new SERD (LSZ102) with alpelisib in endocrine-resistant BC patients (Table 3).

**Multiple targeting of the PI3K/AKT/mTOR pathway**

Although PI3Ki effectively block PI3K and down-regulate its stimuli to cell proliferation, BC cells are able to reactivate PI3K/AKT/mTOR pathway signalling through the activation of downstream effectors such as AKT and mTOR, and thereby develop resistance to PI3Ki [29]. A potential strategy to overcome this resistance mechanism is the concomitant inhibition of multiple targets on the PI3K/AKT/mTOR pathway, which can be achieved by the combination of different inhibitors, or by agents that block multiple kinases [2, 60]. Thus, ongoing studies are evaluating the blockade of PI3K/AKT/mTOR signalling at multiple sites as a way to overcome treatment resistance in BC (Table 3).

**With antiandrogens**

PIK3CA-mut can be found in up to 40% of BC patients whose tumours express androgen receptors (AR), and the expression of AR is higher in BC that harbour mutations in the PI3K kinase domain than in PIK3CA wild-type BC [69, 70]. In preclinical models of luminal and triple-negative BC cells, there is a significant cross-talk between the PI3K and the AR pathways, with the activation of the AR inducing PTEN expression and rendering BC cells more sensitive to PI3K inhibition [71]. In cell lines and xenograft models of triple-negative BC cells that express AR, a synergy between the combination of PI3Ki and AR inhibitors has been demonstrated, with the combination exerting a more robust antitumoural effect than each agent alone [69]. Based on this preclinical data, the combination of the α-selective PI3Ki alpelisib
| Trial | Phase | Nb. of pts | Inclusion criteria | Treatment arms (control versus experimental) | Results (control versus experimental)* | Comments |
|-------|-------|------------|-------------------|---------------------------------------------|----------------------------------------|----------|
| Neoadjuvant setting | With anti-HER2 therapy (and chemotherapy) | NeoPHOEBE [63] | II | 50 | HER2+, tumour diameter >2 cm by clinical examination and/or >1.5 cm by ultrasound/MRI | Paclitaxel+trastuzumab versus paclitaxel+trastuzumab+buparlisib | pCR rate: 32% 40%  P=0.811 | EFS: Not reported; trial stopped earlier due to an excess in liver toxicity in the experimental arm |
| Metastatic setting | With CDK4/6 inhibitors | Juric [47] | I | 36 | ER+/HER2-- ABC | Cohort 3: alpelisib+ribociclib+letrozole | ORR: 7%, SD: 22% | All grade AE > 35% of patients: nausea, hyperglycaemia, neutropenia and fatigue |
| | With chemotherapy | PIPA [16] | I | 35 | PIK3CA-mut ABC | Palbociclib+taselisib+fulvestrant (ER+ cohort) | ORR L cohort: 33% ORR F cohort: 30% | – |
| | With anti-HER2 therapy (± chemotherapy) | PEGGY [11] | II | 183 | ER+/HER2-- ABC; prior CT not allowed with the exception of capecitabine or mTORi | Placebo+paclitaxel versus pictilisib plus paclitaxel | mPFS: 7.8 versus 8.2 m HR 0.95 (95% CI 0.62–1.46) | Pictilisib did not improve PFS also in the PIK3CA-mut subgroup |
| | | BELLE-4 [9] | II | 416 | First-line therapy in HER2-- ABC Stabilisation according to PI3K pathway activation and ER status | Placebo+paclitaxel versus buparlisib+paclitaxel | mPFS: 9.2 versus 8.0 m HR 1.18 (95% CI 0.82–1.68) | Tendency for better mPFS for PI3K activated population with buparlisib. Trial halted before entering phase III |
| | | McRee [49] | I | 25 | ABC for which capecitabine was deemed a reasonable option | Escalating doses of buparlisib (three levels) and capecitabine (two levels) | NA | Buparlisib MTD: 100 mg daily; capecitabine MTD: 1000 mg/m² twice daily |
| | | Sharma [21] | I/II | 43 | HER2 -- ABC; >6 months from prior solvent-based taxane | Alpelisib+nab-paclitaxel | PI3K activated: mPFS 13 m PI3K inactivated: mPFS 7 m ORR in ER+ ABC: 60% | PI: Alpelisib RP2D: 350 mg/day |
| | | Cruz-Zambriano [50] | I | 64 | Refractory solid tumours, including 11 HER2-- and 11 HER2+ ABC | Arm 2. buparlisib+paclitaxel (solid tumours) | ORR arm 2: 17% ORR arm 4: 27% | Buparlisib MTD arm 2 and 4: 100 mg/day |
| | | Rodon-Ahnerf | I | 46 | Refractory solid tumours, including 11 HER2-- and 11 HER2+ ABC | Arm 1. dactolisib+paclitaxel (solid tumours) | ORR arm 1: 9% ORR arm 3: 55% | Dactolisib MTD arm 1 and 3: 800 mg/m²/week |

*Continued*
| Trial                        | Phase | Nb. of pts | Inclusion criteria                        | Treatment arms (control versus experimental)                          | Results (control versus experimental) | Comments                          |
|-----------------------------|-------|------------|-------------------------------------------|-----------------------------------------------------------------------|---------------------------------------|------------------------------------|
| Saura [52] and Pistilli [53] | I/II  | 68         | HER2+ ABC after failing trastuzumab        | Phase I: escalating doses of buparlisib+trastuzumab; phase II: RP2D found in phase I for the combination | ORR: 10%                              | Buparlisib RP2D: 100 mg/day; Trastuzumab RP2D: 2 mg/kg q7days. Deemed inactive |
| Tolaney [54]             | I/II  | 42         | HER2+ ABC after failing trastuzumab        | Arm 1: pilaralisib+trastuzumab; Arm 2: pilaralisib+trastuzumab+paclitaxel | NA                                    | Pilaralisib MTD: 400 mg; did not enter phase II |
| Shah [55]                  | I     | 10         | PIK3CA-mut HER+ ABC after progression under pertuzumab and T-DM1 | Alpelisib+LIM7 16+trastuzumab | DCR: 79%                              | Combination too toxic to warrant further testing |
| PiliHER2[56]              | I     | 25         | HER2+ ABC after progression under trastuzumab | Escalating doses of buparlisib+lapatinib | mPFS: 6 m                              | RP2D: buparlisib 80 mg/day; MTD: 250 mg/day |
| Jain [57]                  | I     | 17         | HER2+ ABC after a taxane+trastuzumab-based therapy | De-escalating doses of alpelisib combined with T-DM1 | mPFS: 7.6 m                             | MTD: 1000 mg/day; Phase II planned |
| Metzger Filho [58]        | I     | 26         | HER+ ABC regardless of previous lines of anti-HER2 therapy | Cohort A: taselisib + T-DM1 | mPFS: 15.5 m                           | No DLT in tested doses |
| Schoffski [59]            | I     | 69         | ABC treated with ≤2 lines of CT (part 1 and 2) ER + ABC treated with ≤1 line of CT or ≤2 lines of ET | Part 1: pictilisib+paclitaxel+bevacizumab; Part 2A: pictilisib+paclitaxel → → bevacizumab (2B) → → trastuzumab (2C); Part 3: pictilisib + letrozole | mPFS: 5.2 m; 2B: 7.5 m; 2C: 14.8 m | Pictilisib 260 mg selected as RP2D but further development of the drug halted |
| PANTHERA [46]            | I/II  | 12         | HER2+ ABC progressing after ≥1 line of trastuzumab or T-DM1 | Copanlisib+trastuzumab | ORR: 0% DCR: 75%                       | RP2D for copanlisib: 60 mg Will enter phase II |
| With mTOR inhibitors Baselga [60] | I | 7          | ER+/HER2 – ABC                             | Escalating doses of alpelisib+everolimus+exemestane | NA                                    | MTD for alpelisib: 200 mg         |

*Wherever applicable.

bSame study reported in 2 separated abstracts, one for each pair of arms.

ABC, advanced breast cancer; AE, adverse events; CI, confidence interval; CT, chemotherapy; DCR, disease control rate; EFS, event-free survival; ER, estrogen receptor; ET, endocrine therapy; HR, hazard ratio; m, months; mt, mutant; MTD, maximum tolerated dose; NA, not available; ORR, overall response ratio; pCR, pathological complete response; mPFS, median progression-free survival; RP2D, recommended phase II dose; SD, stable disease; T-DM1, ado-trastuzumab emtansine.
## Table 3. Ongoing phase I/II/III combination trials with PI3K inhibitors in the early and metastatic estrogen-receptor positive breast cancer settings

| ClinicalTrials.gov identifier (Trial name) | Phase | Design | Patient population | Number of patients | Treatment arms | Objectives |
|------------------------------------------|-------|--------|---------------------|--------------------|----------------|------------|
| PI3K inhibitors + CDK4/6 inhibitors (+ dual PI3K/mTOR inhibitors) | I/II | Randomized, open-label, three-arm trial | ER+/HER2−, stage II/III | 102 | Arm A: copanlisib + letrozole; Arm B: copanlisib + palbociclib + letrozole; Arm C: palbociclib + letrozole + copanlisib after breast biopsy on day 14 | Primary: change in Ki67 (baseline to 2 weeks); Secondary: pCR, ORR, AE, among others |
| NCT02626507 | I | Open-label, single arm trial | ER+/HER2−, stage I–IV, intended for surgery of the primary tumour | 18 | Gedatolisib + fulvestrant + palbociclib (+ goserelin if pre-menopausal) | Primary: incidence of treatment-related AE; Secondary: pCR |
| Metastatic | PI3K inhibitors + CDK4/6 inhibitors | | | | | |
| NCT03939897 | I/II | Open label, non-randomized, two-arm trial | ER+/HER2−, endocrine-resistant ABC | 194 | Copanlisib + abemaciclib + fulvestrant versus abemaciclib + fulvestrant | Primary: DLT (phase I), PFS (phase II); Secondary: ORR, CBR, OS, among others |
| NCT03128619 | lb | Single-arm, open-label trial | ER+/HER2−, ABC, first-line treatment | 102 | Copanlisib + palbociclib + letrozole | Primary: incidence of DLT; Secondary: ORR, AE, PK |
| NCT02088684 | lb/II | Randomized, open-label, three-arm trial | ER+/HER2− ABC, ≤2 lines of chemotherapy in phase I and ≤1 line in phase II | 70 | Ribociclib + fulvestrant + buparlisib versus ribociclib + alpelisib + fulvestrant versus ribociclib + fulvestrant | Primary: DLT (phase II), PFS (phase II); Secondary: safety; ORR, DoR, OS; Phase II portion not opened |
| NCT02154776 (LeeBLet) | I | Single-arm, open-label trial | ER+/HER2− ABC, ≤2 lines of chemotherapy | 13 | Buparlisib + ribociclib + letrozole | Primary: DLT, safety of the combination; Secondary: DCR, PFS, PK |
| PI3K inhibitors + chemotherapy | | | | | | |
| NCT03218826 | I | Single-arm, open-label trial | PTEN or PIK3CB mutated, HER2− ABC, among other tumours | 58 | AZD8186 + docetaxel | Primary: MTD and RP2D; Secondary: ORR, CBR, drug-drug interactions |
| PI3K inhibitors + anti-HER2 agents (± chemotherapy) | | | | | | |
| NCT01285466 | lb | Open label, non-randomized, multi-arm trial | HER2+ ABC eligible for paclitaxel and trastuzumab (for the cohort of breast cancer patients) | 110 | Dactolisib + paclitaxel + trastuzumab or Buparlisib + palbociclib + trastuzumab | Primary: DLT; Secondary: safety, PK, efficacy, among others |
| NCT02390427 | lb | Non-randomized, open label, four-arm trial | HER2+ ABC with previous anti-HER2 treatment | 76 | Arm A: taselisib + T-DM1; Arm B: taselisib + T-DM1 + pertuzumab; Arm C: taselisib + trastuzumab + pertuzumab; Arm D: equal to arm C + palitaxel | Primary: MTD of taselisib in each arm; Secondary: CBR, PFS, OS, AE, among others |
| NCT00928330 | I | Non-randomized, open-label trial | HER2+ ABC, after progressing on trastuzumab-based treatment | 57 | Pictilisib + T-DM1; Pictilisib + trastuzumab | Primary: change in cardiac function, among others; Secondary: PK, PFS, among others |
| ClinicalTrials.gov identifier (Trial name) | Phase | Design | Patient population | Number of patients | Treatment arms | Objectives |
|------------------------------------------|-------|--------|--------------------|-------------------|----------------|------------|
| NCT03765983                             | II    | Single arm, open label trial | HER+ ABC with CNS involvement | 47                | GDC-0084 + trastuzumab | Primary: ORR in the CNS Secondary: CBR, PFS, OS, AE, among others |
| PANTHERA [46]                           | Ib    | Single arm, open label trial | HER2+ ABC after trastuzumab and paclitaxel | 24                | Copanlisib + T-DM1 | Primary: MTD for copanlisib Secondary: safety, efficacy, among others |
| NCT NA                                  | I     | Single arm, open label trial | PIK3CA mutated HER2+ ABC, after > 2 lines of treatment, including trastuzumab | 48                | MEN1611 + trastuzumab ± fulvestrant | Primary: MTD and RP2D Secondary: treatment emergent AE, PFS, OS |
|                                          |       |        |                    |                   |                |            |
| Multiple targeting of the PI3K/AKT/mTOR pathway |       |        |                    |                   |                |            |
| NCT03006172                             | I     | Open label, non-randomized, multi-arm trial | For breast cancer cohorts: ER+/HER2 – PIK3CA-mutant, progressing on previous therapy | 196               | GDC-0077 + palbociclib + letrozole or fulvestrant, among others | Primary: DLT, RP2D Secondary: PK, ORR, PFS, among others |
| NCT02684032                             | I     | Open label, non-randomized, multi-arm trial | ER+/HER2 – ABC in various settings | 148               | Gedatolisib + palbociclib + fulvestrant or letrozole | Primary: DLT, ORR Secondary: tumour response, DoR, among others |
| NCT02077933                             | Ib    | Open label, non-randomized, crossover assignment trial | All solid tumours; Cohort of ABC patients with no standard therapy available | 79                | Alpelisib + everolimus; Alpelisib + everolimus + exemestane; Alpelisib + exemestane | Primary: DLT, safety and tolerability Secondary: PK, PFS, DoR, CBR, ORR |
| NCT01899053                             | I     | Open label, non-randomized, parallel assignment trial | All solid tumours except brain primary with no standard therapy available | 101               | Sapanisertib + MLN117 | Primary: incidence of AE, PK Secondary: ORR, DoR |
| NCT01248494                             | Ib    | Open label, randomized, multi-arm trial | ER+ ABC, no limit on prior number of therapies; HR+/HER2+ patients must have failed trastuzumab | 72                | Dactolisib + letrozol; Buparlisib + letrozol; Intermittent buparlisib + letrozol | Primary: MTD of buparlisib and dactolisib Secondary: PFS, ORR |
| NCT01082068                             | I/II  | Open label, non-randomized, parallel assignment trial | HR+/HER2- ABC refractory to a non-steroidal aromatase inhibitor | 72                | Arm 1: pilaralisib + letrozol; Arm 2: voxtalisib + letrozol | Primary: MTD for both drugs with letrozol in phase I, PFS in phase II Secondary: CBR, PK |

Continued
| ClinicalTrials.gov identifier (Trial name) | Phase | Design | Patient population | Number of patients | Treatment arms | Objectives |
|------------------------------------------|-------|--------|--------------------|--------------------|---------------|------------|
| NCT02723877 (PIQHASSO)                  | I/II  | Single arm, open label trial | HER2−: ABC previously treated with an anthra-cycline and a taxane | 41 | PQR309 + eribulin | Primary: safety, CBR  
Secondary: PK, ORR, DOR, PFS, among others |
| P3K inhibitors + selective estrogen receptor degraders (SERD) |       |        |                    |                   |               |            |
| NCT02734615                             | Ib    | Open label, randomized, parallel assignment trial | ER+/HER2−: ABC | 312 | Arm A: LSZ102  
Arm B: LSZ102 + ribociclib  
Arm C: LSZ102 + alpelisib | Primary: DLTs (dose escalation), safety of LSZ102 and LSZ102 + ribociclib (dose expansion)  
Secondary: ORR, DoR, PFS, PK |
| P3K inhibitors + androgen receptor inhibitor |       |        |                    |                   |               |            |
| NCT03207529                             | I     | Single arm, open-label trial | ER+ or −, HER2−: AR+, PTEN + ABC | 28 | Alpelisib + enzalutamide | Primary: MTD  
Secondary: PFS and CBR at 16 weeks |

*Duplicated study as it comprises two phases in different settings.

ABC, advanced breast cancer; AE, adverse events; AR, androgen receptor; CBR, clinical benefit rate; CNS, central nervous system; DCR, disease control rate; DLT, dose limiting toxicities; DoR, duration of response; ER+, estrogen receptor-positive; ER−, estrogen receptor-negative; MTD, maximum tolerated dose; OS, overall survival; ORR, objective response rate; pCR, pathologic complete response; PFS, progression-free survival; PK, pharmacokinetic parameters; RP2D, recommended phase II dose.
with enzalutamide (an AR antagonist) is currently being evaluated in a phase I study in HER2-negative metastatic BC patients whose tumours express both AR and PTEN by immunohistochemistry (Table 3).

**Conclusion**

Despite intense research efforts, so far, only PIK3CA mutations have proved to have a predictive value for treatment with α-selective and β-sparing PI3Ki in the advanced setting. Thus, its assessment has recently entered clinical practice. Even so, a composite biomarker, which could more accurately assess PI3K/AKT/mTOR pathway activation, would be the preferred approach. This question is even more pressing as new drug combinations with PI3Ki are being developed and may enter clinical practice in the future, making better treatment tailoring an urgent need.

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