Optimal targeting of PI3K-AKT and mTOR in advanced oestrogen receptor-positive breast cancer

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The growing availability of targeted therapies for patients with advanced oestrogen receptor-positive breast cancer has improved survival, but there remains much to learn about the optimal management of these patients. The PI3K–AKT and mTOR pathways are among the most commonly activated pathways in breast cancer, whose crucial role in the pathogenesis of this tumour type has spurred major efforts to target this pathway at specific kinase hubs. Approvals for oestrogen receptor-positive advanced breast cancer include the PI3K inhibitor alpelisib for *PIK3CA*-mutated tumours, the AKT inhibitor capivasertib for tumours with alterations in *PIK3CA*, *AKT1*, or *PTEN*, and the mTOR inhibitor everolimus, which is used irrespective of mutation status. The availability of different inhibitors leaves physicians with a potentially challenging decision over which of these therapies should be used for individual patients and when. In this Review, we present a comprehensive summary of our current understanding of the pathways and the three inhibitors and discuss strategies for the optimal sequencing of therapies in the clinic, particularly after progression on a CDK4/6 inhibitor.

Lancet Oncol 2024; 25: e139–51

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Introduction

The PI3K-AKT and mTOR signalling pathways have crucial functions in physiological cellular homoeostasis and metabolism. These pathways are constitutively activated by genetic alterations in approximately 50% of oestrogen receptor (ER)-positive, HER2-negative breast cancer,[1–3] which is a driver of resistance to endocrine-based treatment. Moreover, these pathways are likely to be activated in the majority of ER-positive tumours without genetic alterations, due to upstream receptor tyrosine kinase (RTK) signalling. Although PI3K-AKT and mTOR signalling are attractive targets for therapy, the physiological functions of these pathways make therapeutic index the crucial concern, with approaches to maximise both drug tolerance and therapeutic benefit being paramount.

Treatment of ER-positive metastatic breast cancer is rapidly evolving, with the continued growth in precision medicine and reduced use of chemotherapy in early lines of treatment. CDK4/6 inhibition in combination with endocrine therapy is the gold standard first-line treatment, improving both overall survival and progression-free survival compared with endocrine therapy alone.[4–6] Most patients eventually have disease progression, at which point multiple competing treatment options present a clinical challenge, with inhibitors of the PI3K, AKT, or mTOR kinases being an option, depending on local approval and reimbursement protocols. Here, we review inhibitors of the PI3K-AKT and mTOR pathways, contrasting their mechanisms of action, clinical evidence, and drug tolerance. We discuss selection strategies for individual patients in the context of changing management in the era of precision oncology.

The PI3K-AKT and mTOR pathways

The PI3K-AKT and mTOR signalling pathways are key regulators of normal cellular growth, proliferation, metabolism, and survival.[7] Although these pathways are often referred to as a single pathway, this simplification hampers our perception of how these proteins are activated in breast cancer as well as how best to target them in the clinic (figure 1). PI3K, AKT, and mTOR are major signalling nodes. In terms of activity, PI3K and AKT are more closely linked with each other than with mTOR, with mTOR ultimately having diverse inputs in addition to PI3K-AKT signalling (figure 2).

PI3Ks are a diverse group of lipid kinases that are located at intracellular and plasma membranes and divided into three classes (I, II, and III).[8] In breast cancer, PI3K signalling commonly refers to class I PI3Ks, which consist of a p85 regulatory subunit and p110 catalytic subunit and are activated by RTKs, G protein-coupled receptors, and activated RAS.[9,10] Two homologous catalytic subunits of class I PI3Ks, p110α (encoded by *PIK3CA*) and p110β (encoded by *PIK3CB*), are broadly expressed in breast cancer, whereas other p110 subunits (p110γ and p110δ) are only expressed in other cell types.[9]

On activation, the PI3Ks catalyse the phosphorylation of plasma membrane lipid PIP2 to PIP3, which promotes the recruitment and activation of AKT and PDK1.[10] PTEN, the main negative regulator of PI3K signalling, modulates this pathway through the dephosphorylation of PIP3 to PIP2[11] (figure 1).

PDK1 and AKT activity are tightly regulated by PI3K activity, with AKT the key canonical effector of PI3K signalling. AKT binds to PIP3, recruiting it to the plasma membrane, and is further activated by PDK1 phosphorylation.[12,13] AKT, a serine-threonine kinase with three isoforms (AKT1, AKT2, and AKT3), is the central mediator of this pathway, modulating the function of more than 100 substrates, including MDM2, GSK3β, and the forkhead family of transcription factors.[14] PI3K can signal independently of AKT, through SGK3, promoting downstream signalling,[15] although the relevance of this particular pathway to breast cancer is uncertain.

mTOR is an atypical serine-threonine kinase that exists in two structurally and functionally distinct complexes; namely, mTORC1, with raptor and PRAS40, and

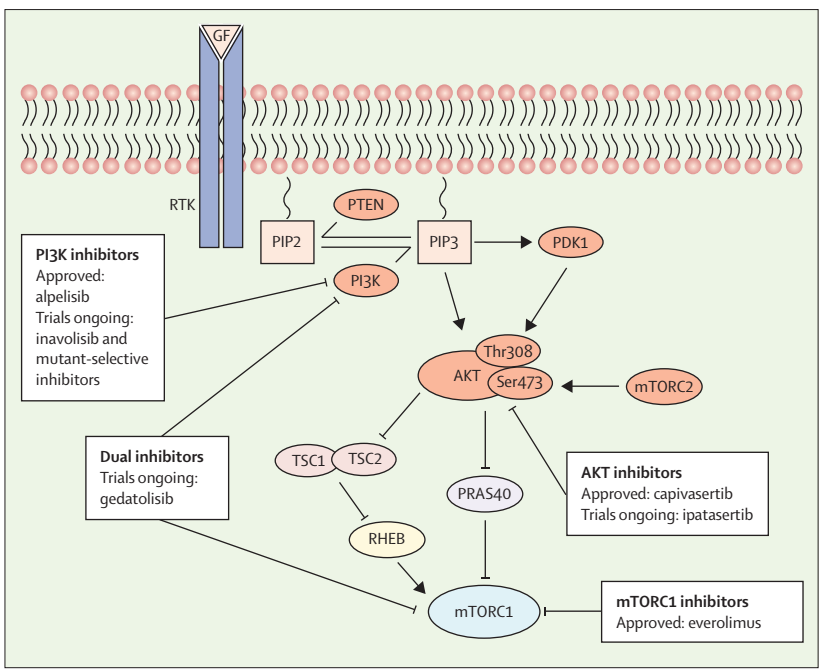

Figure 1: **PI3K-AKT and mTOR pathways**
On activation, PI3K catalyses the phosphorylation of PIP2 to PIP3. PTEN is the main negative regulator of PI3K signalling, through the dephosphorylation of PIP3 to PIP2. AKT binds to PIP3, recruiting it to the plasma membrane, which allows PDK1 to phosphorylate AKT at Thr308, within the kinase domain. For full activation of AKT, a second phosphorylation by mTORC2 is required at the regulatory domain Ser473. AKT modulates mTORC1 activity via the phosphorylation and inhibition of TSC1 and TSC2, which increases RHEB activity, in turn activating mTORC1. Mutant-selective inhibitors are listed in table 1. GF=growth factor. RTK=receptor tyrosine kinase.

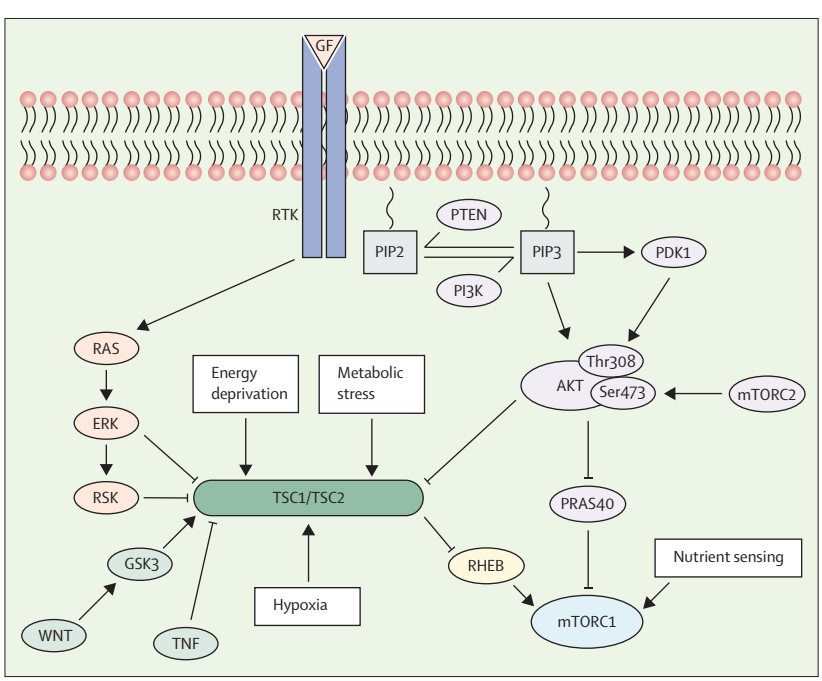

Figure 2: **Diversity of inputs into mTORC1**
mTOR has multiple diverse inputs in addition to AKT signalling, including inputs from GFs, amino acids, cellular energy status, and cellular stress. GF=growth factor. RTK=receptor tyrosine kinase.

mTORC2, with rictor, mSIN1, and protor-1/2.[16] In breast cancer, mTOR signalling generally refers to mTORC1, which is inhibited by everolimus. mTORC2 is not inhibited by everolimus, and, importantly, mTORC2 phosphorylates AKT to promote AKT activation.[13] AKT modulates mTORC1 activity via the phosphorylation and inhibition of TSC1 and TSC2, in turn increasing the activity of RHEB, which activates mTORC1.[17] mTORC1 has multiple other inputs (figure 2).

PI3K-AKT and mTORC1 have several distinct cellular roles. For example, activation of PI3K and AKT promotes glucose metabolism,[18,19] whereas mTORC1 controls cap-dependent translation and plays a role in normal T-cell homoeostasis.[20,21]

Activation of the PI3K-AKT and mTOR pathways in breast cancer

Activation of the PI3K-AKT and mTOR pathways is a hallmark of cancer. Including genetic activation of upstream receptors, up to 70% of breast cancers are estimated to have mutations that lead to pathway hyperactivation.[22] Alterations in this pathway can be present in the original primary cancer or acquired as a result of adaption to previous therapy.[23,24]

Genetic activation

Mutations in *PIK3CA* (which encodes p110α) are observed in 35–40% of ER-positive, HER2-negative breast cancers.[1,25] The majority of *PIK3CA* somatic mutations are located in the helical domain (1624G>A [Glu542Lys] or 1633G>A [Glu545Lys] in exon 9) or kinase domain (3140A>G [His1047Arg] or 3140A>T [His1047Leu] in exon 20) of p110α.[25] Mutations outside these locations have been less explored in the clinical setting, as we discuss later. The majority of *PIK3CA* mutations are present in the primary tumour and subsequent recurrence, although a small minority of *PIK3CA* mutations are acquired through previous endocrine and CDK4/6 inhibitor therapy.[23] Up to 25% of advanced cancers with *PIK3CA* mutations have a second mutation on the same allele (ie, in *cis*). Tumours with double *PIK3CA* mutations have a more active PI3K enzyme and might be more responsive to PI3K inhibitors than those with single mutations.[26] These second mutations are frequently acquired as a result of APOBEC editing and often occur at distinct amino acids to the initial activating mutations.[27] Although more frequently seen in triple-negative breast cancers, mutations in the p85 regulatory subunit (*PIK3R1*) have been observed rarely in ER-positive breast cancer.[28,29]

Loss-of-function *PTEN* mutations and homozygous deletion of *PTEN* are observed in 5–10% of breast cancers.[2,30,31] Truncating mutations are highly likely to result in loss of PTEN function, whereas only a subset of missense mutations result in loss of PTEN function.[32,33] Loss-of-function *PTEN* mutations might be acquired through CDK4/6 inhibitor therapy as a mechanism of

resistance.[34] Loss of PTEN strongly activates AKT, in part through activation of p110β signalling, limiting the activity of drugs that target p110α.[35,36]

Activating mutations of AKT1 occur in about 2–3% of primary ER-positive breast cancer and about 5–7% of advanced cancers.[3,37,38] Approximately 80% of AKT mutations occur at Glu17Lys, which increases AKT activity independent of PI3K by promoting AKT localisation to the plasma membrane.[39,40] Additional AKT mutations include Leu52Arg, Asp32Tyr, and Lys39Asn, and rare mutations occur in AKT2, at Glu17Lys.[41] AKT activation, as well as activation of the whole pathway, has been shown to confer resistance to endocrine agents, PARP inhibitors, and chemotherapy.[42–44]

Non-genomic activation

PI3K-AKT and mTOR activation can occur independent of genetic mutations. ER-positive breast cancers can upregulate PI3K-AKT and mTOR signalling as a key mechanism of acquired resistance to oestrogen deprivation.[45] PI3K signalling is also strongly activated by amplified and overexpressed HER2 signalling.[46] Crosstalk between the ER and PI3K pathways is complex; PI3K inhibition leads to upregulation of ER signalling and increases ER dependence in ER-positive breast cancer.[47]

Mutations in mTOR are essentially not observed in breast cancer; rather, mTORC1 is activated by upstream PI3K-AKT signalling, MAPK signalling, and other activators and as a mechanism of resistance to endocrine

	Patient mutation status	Line of treatment	Combined therapy	Primary endpoint	Phase of development	Trial name	Trial registration number
PI3K inhibitors							
Inavolisib (trial 1)	*PIK3CA*	First line, advanced*	Inavolsilib plus palbociclib plus fulvestrant *vs* placebo plus palbociclib, plus fulvestrant	Progression-free survival	3	INAVO120	NCT04191499
Inavolisib (trial 2)	*PIK3CA*	After progression on CDK4/6 inhibitor in advanced setting†	Inavolisib plus fulvestrant *vs* alpelisib plus fulvestrant	Progression-free survival	3	INAVO121	NCT05646862
Alpelisib	*PIK3CA* DNA non-suppression C2D1	First line, advanced*	Alpelisib plus fulvestrant *vs* palbociclib plus fulvestrant	Progression-free survival	2	SAFIR 03	NCT05625087
TOS-358	*PIK3CA*	Not specified	Monotherapy	Dose-limiting toxicities, adverse events	1	TOS-358-001	NCT05683418
LOXO-783‡	*PIK3CA* (3140A>G [His1047Arg])	Arm dependent§	Multiple treatment groups¶	Dose-limiting toxicities	1	PIKASSO-01	NCT05307705
STK-478‡	PIK3CA (His1047Xxx)‖	Not specified	STX-478 as monotherapy or in combination with fulvestrant	Dose-limiting toxicities	1/2	SCORPION	NCT05768139
RLY-2608‡	*PIK3CA*	After endocrine therapy and CDK4/6 inhibitor, maximum one line of chemotherapy in advanced setting	Multiple treatment groups**	Dose-limiting toxicities, adverse events	1/2	ReDiscover	NCT05216432
Pan-AKT inhibitor							
Ipatasertib	ctDNA non-suppression C1D15	After endocrine therapy,†† maximum one line of chemotherapy in advanced setting	Ipatasertib plus palbociclib plus fulvestrant *vs* palbociclib plus fulvestrant	Progression-free survival	2	FAIM	NCT04920708
Capivasertib	Mutation not required	After endocrine therapy‡‡	Capivasertib plus fulvestrant plus CDK4/6inhibitor (palbociclib or ribociclib) *vs* fulvestrant plus CDK4/6 inhibitor (palbociclib or ribociclib)	Dose-limiting toxicities, adverse events, progression-free survival	1b/3	CAPItello-292	NCT04862663
mTOR inhibitor							
RMC-5552§§	Mutation not required	Not specified	Monotherapy	Dose-limiting toxicities, adverse events	1	RMC-5552-001	NCT04774952
Dual inhibitor							
Gedatolisib¶¶	Mutation not required	After CDK4/6 inhibitors plus aromatase inhibitor in advanced setting	Multiple treatment arms‖‖	Progression-free survival	3	VIKTORIA-1	NCT05501886

ctDNA=circulating tumour DNA. *Patients must have progressed on or within 12 months of completion of adjuvant endocrine therapy, with no previous systemic therapy for advanced disease. †Maximum two previous lines of systemic treatment in advanced setting; CDK4/6i-based therapy does not need to be the last treatment received before study entry. ‡Mutant-selective. §Between two and five lines of systemic treatment allowed, dependent on treatment arm. ¶Arm A: LOXO-783 plus physician's choice (fulvestrant or imlunestrant or aromatase inhibitor); arm B: LOXO-783 plus abemaciclib physician's choice (fulvestrant or imlunestrant or aromatase inhibitor); arm C: LOXO-783 plus fulvestrant; arm D: LOXO-783 plus paclitaxel; arm E: LOXO-783 monotherapy; arm F: multiple dose levels of LOXO-783 with fulvestrant. ‖Other kinase domain mutations. **Arm A: RLY-2608 monotherapy; Arm B: RLY-2608 plus fulvestrant; Arm C: RLY-2608 plus fulvestrant plus palbociclib; arm D: RLY-2608 plus fulvestrant plus ribociclib (400 mg); arm E: RLY-2608 plus fulvestrants plus ribociclib (600mg). ††Patients must have progressed on or within 1 month of previous first-line endocrine therapy for advanced disease or have relapsed on or within 12 months of completing adjuvant endocrine therapy. ‡‡ Progression while on or within 12 months of completing adjuvant endocrine therapy, maximum one line of chemotherapy in metastatic setting. §§mTORC1 inhibitor. ¶¶Inhibitor of class I PI3K and mTOR. ‖‖Arm A: wild-type PIK3CA, gedatolisib plus fulvestrant plus palbociclib; arm B: wild-type PIK3CA, gedatolisib plus fulvestrant; arm C: wild-type PIK3CA, fulvestrant; arm D, mutated PIK3CA, gedatolisib plus fulvestrant plus palbociclib; arm E: mutated PIK3CA, alpelisib plus fulvestrant; arm F: mutated PIK3CA, gedatolisib plus fulvestrant.

Table 1: **Selected ongoing trials of novel PI3K–AKT and mTOR pathway inhibitors for metastatic ER-positive, HER2-negative breast cancer**

therapy.[48] The extent to which mTOR signalling in endocrine resistance depends on AKT activation is unknown.

Reported clinical trials in advanced breast cancer

Pan-PI3K inhibitors

First-generation PI3K inhibitors (pan-PI3K inhibitors) targeted all four isoforms of class I PI3Ks (α, β, γ, and δ). The lack of selectivity of these drugs for p110α resulted in high rates of adverse events, which limited the ability to give these drugs at a sufficiently high dose.

Buparlisib inhibits all class I isoforms and somatically mutant p110α. The BELLE-2[49] (n=1147) and BELLE-3[50] (n=432) phase 3 trials evaluated buparlisib in combination with fulvestrant in postmenopausal women with endocrine-resistant, metastatic, ER-positive, HER2-negative breast cancer. In BELLE-2, without previous everolimus, buparlisib modestly improved median progression-free survival overall (6·9 months, [95% CI 6·8–7·8] with buparlisib *vs* 5·0 months [4·0–5·2] with placebo; hazard ratio [HR] 0·78, 95% CI 0·67–0·89; p=0·0002). In patients with *PIK3CA* mutations in circulating tumour DNA (ctDNA), median progression-free survival was 7·0 months (95% CI 5·0–10·0) with buparlisib versus 3·2 months (2·0–5·1) with placebo (HR 0·58, 95% CI 0·41–0·82; p=0·001), with no benefit in patients without *PIK3CA* mutations in ctDNA (1·05, 0·82–1·34; p=0·642).[49] In BELLE-3, with previous everolimus, buparlisib improved median progression-free survival in patients with *PIK3CA* mutations in ctDNA (4·2 months [95% CI 2·8–6·7] *vs* 1·6 months [1·4–2·8]; HR 0·46, 95% CI 0·29–0·73, p=0·0003) with a more modest improvement in patients without *PIK3CA* mutations in ctDNA (3·9 months [95% CI 2·8–4·3] *vs* 2·7 months [1·5–3·6]; HR 0·73, 95% CI 0·53–1·00; p=0·026).[50] The BELLE series of trials was, therefore, important in showing that buparlisib was predominantly only effective in *PIK3CA*-mutated cancer and that there was no cross-resistance between buparlisib and everolimus. However, the adverse effects of buparlisib were unfavourable. In BELLE-2, 222 (39%) of 576 patients discontinued the study drug early. Additionally, buparlisib crosses the blood–brain barrier, and psychiatric issues such as anxiety, depression, and suicidal ideation were reported.[49,50] Thus, buparlisib was not implemented in clinical practice. In preclinical studies, buparlisib was noted to interfere with microtubule polymerisation, which might have contributed to the adverse effects.[51]

Pictilisib, an oral drug that also inhibits all class I isoforms, was evaluated in FERGI[52] (n=168), a phase 2 trial in postmenopausal women with ER-positive, HER2-negative disease resistant to aromatase inhibitors. No difference in median progression-free survival was seen in the intervention group, regardless of *PI3KCA* mutation status (intention-to-treat population HR 0·74, 95% CI 0·52–1·06; p=0·096). As with buparlisib, this drug was limited by its toxicity profile, with 53 (60%) of 89 patients experiencing grade 3 or worse adverse events.[52]

Isoform-specific PI3K inhibitors

Taselisib, the first of the selective inhibitors, was designed to inhibit p110α and not p110β, with greater selectivity for mutant than wild-type PI3Kα. However, taselisib also inhibited p110δ and p110γ isoforms, which might have contributed to the failure of this drug in the clinic. SANDPIPER[53] (n=516), a phase 3 trial, evaluated taselisib plus fulvestrant versus fulvestrant in postmenopausal women with ER-positive, HER2-negative, *PIK3CA*-mutated advanced breast cancer after progression on aromatase inhibitors. A small improvement in progression-free survival with taselisib versus placebo was observed in *PIK3CA*-mutant tumours (7·4 months *vs* 5·4 months; HR 0·70, 95% CI 0·56–0·89; p=0·0037). Grade 3–4 adverse events occurred in 206 (49·5%) of 416 patients in the taselisib group; 48 (12%) of patients in the taselisib group had grade 3–4 diarrhoea.[53] In particular, patients developed late-onset diarrhoea and colitis, thought to reflect immune dysregulation through inhibition of p110δ, and further investigation of taselisib was discontinued.

Alpelisib is the first oral α-selective PI3K inhibitor, selectively inhibiting p110α with approximately 50 times more potency than other isoforms. This drug was approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2019 for use in combination with fulvestrant for postmenopausal women and men with ER-positive, HER2-negative, *PIK3CA*-mutated advanced or metastatic breast cancer after aromatase inhibitors. The phase 3 SOLAR-1 trial[54] showed an improved median progression-free survival of 11·0 months (95% CI 7·5–14·5) versus 5·7 months (3·7–7·4) for alpelisib plus fulvestrant for patients with *PIK3CA* mutations (HR 0·65, 95% CI 0.50–0·85; p<0·001). Alpelisib did not show an improvement in median progression-free survival in patients with wild-type *PIK3CA* tumours (0·85, 0·58–1·25, posterior probability of HR <1·0=79·4%).[54] The key secondary endpoint of overall survival in patients with *PIK3CA* mutations did not meet statistical significance (39·3 months [95% CI 34·1–44·9] for alpelisib plus fulvestrant *vs* 31·4 months [26·8–41·3] for placebo plus fulvestrant; HR 0·86, 95% CI 0·64–1·15; p=0·15).[55] Grade 3–4 toxicities in patients receiving alpelisib included hyperglycaemia in 104 (36·6%) of 284 patients, rash in 57 (20·1%), and diarrhoea in 19 (6·7%).

AKT inhibitors

Capivasertib is a potent selective inhibitor of all three AKT isoforms, approved by the FDA in 2023 in combination with fulvestrant for postmenopausal women and men with ER-positive, HER2-negative, PI3K-AKT pathway-altered tumours. The phase 2 FAKTION trial[56] recruited postmenopausal women with ER-

positive, HER2-negative advanced breast cancer with previous aromatase inhibitor therapy, treating with capivasertib and fulvestrant or placebo and fulvestrant. In an updated 5-year analysis, the median progression-free survival was 10·3 months (95% CI 5·0–13·4) with capivasertib versus 4·8 months (3·1–7·9) with placebo (HR 0·56, 95% CI 0·38–0·81; p=0·0023). Median overall survival in the capivasertib group was 29·3 months (95% CI 23·7–39·0) versus 23·4 months (18·7–32·7) in the placebo group (HR 0·66, 95% CI 0·45–0·97; p=0·035).[57] An expanded biomarker analysis defined PI3K-AKT pathway-activated cancers as those with activating *PIK3CA* or *AKT1* mutations or *PTEN* truncating mutations and variants known to inhibit PTEN function. Mutation analysis was performed with a combined analysis of archival tissue and baseline plasma ctDNA testing, which could detect both primary clonal and acquired events. In pathway-altered cancers, median progression-free survival was 12·8 months (95% CI 6·6–18·8) on capivasertib versus 4·6 months (2·8–7·9) on placebo (HR 0·44, 95% CI 0·26–0·72; p=0·0014), and median overall survival was 38·9 months (95% CI 23·3–50·7) on capivasertib versus 20·0 months (14·8–31·4) on placebo (HR 0·46, 95% CI 0·27–0·79; p=0·0047). There were no statistically significant differences in progression-free survival or overall survival in the non-pathway-altered subgroup, suggesting benefit was largely restricted to patients with PI3K-AKT pathway-altered tumours.[57] Overall, the toxicity profile was manageable, with grade 3–4 adverse events occurring in 45 (65%) of 60 patients in the capivasertib group and 35 (50%) of 70 patients in the placebo group.[56]

CAPItello-291[58] was the subsequent phase 3 study that evaluated capivasertib and fulvestrant in patients with ER-positive, HER2-negative advanced breast cancer who progressed during or after aromatase inhibitor therapy with or without a CDK4/6 inhibitor. Patients with previous exposure to PI3K or mTOR inhibitors were excluded from this study. Median progression-free survival was improved with the addition of capivasertib in the overall population (7·2 months [95% CI 5·5–7·4]) *vs* 3·6 months [5·5–7·4]; HR 0·60, 95% CI 0·51–0·71; p<0·001), and in the 289 (41%) of 708 patients with PI3K-AKT pathway-altered cancers (defined as in the expanded biomarker analysis in FAKTION), median progression-free survival was 7·3 months (95% CI 5·5–9·0) versus 3·1 months (2·0–3·7; HR 0·50, 95% CI 0·38–0·65; p<0·001). In patients with AKT pathway-unaltered tumours, excluding unknown results, median progression-free survival was 5·3 months (95% CI 3·6–7·3) versus 3·7 months (3·5–5·1; HR 0·79, 95% CI 0·61–1·02). The most frequently reported grade 3–4 adverse events in the capivasertib group were rash, in 43 (12·1%) of 355 patients, diarrhoea, in 33 (9·3%), and hyperglycaemia, in eight (2·3%).[58]

In contrast with FAKTION[56] and CAPItello-291,[58] AKT inhibitors used in combination with paclitaxel chemotherapy have not improved survival outcomes to date. The phase 2 BEECH study[59] evaluated the efficacy of capivasertib combined with paclitaxel in patients with ER-positive advanced breast cancer and no previous chemotherapy exposure for advanced disease; there was no improvement in median progression-free survival overall (HR 0·80, 80% CI 0·6–1·06; p=0·308) in these patients or in the subpopulation with mutant *PIK3CA* (1·11, 0·73–1·68; p=0·760). A similar study, IPATunity130[60] cohort B, investigated ipatasertib, an inhibitor of all AKT isoforms, in combination with paclitaxel in *PIK3CA*-mutated tumours, again with no median progression-free survival benefit (HR 1·00, 95% CI 0·71–1·40; p=0·997). The most probable interpretation of the absence of improvement in progression-free survival in these two studies is the absence of inhibition of the oestrogen receptor and the failure to block its feedback activation that occurs with AKT inhibition.

mTOR inhibitors

mTOR inhibitors were the first compounds developed to target PI3K-AKT-mTOR signalling, with everolimus in combination with exemestane approved by the FDA and EMA in 2012 for patients with ER-positive, HER2-negative advanced breast cancer after progression on an aromatase inhibitor. Everolimus inhibits mTOR through allosteric binding to mTORC1. Results from BOLERO-2[61] (exemestane with or without everolimus), supported by TAMRAD[62] (tamoxifen with or without everolimus), showed benefit of everolimus in postmenopausal women with hormone-refractory, metastatic, ER-positive, HER2-negative breast cancer. Median progression-free survival was improved in both BOLERO-2 (7·8 months *vs* 3·2 months; HR 0·45, 95% CI 0·38–0·54; p<0·0001)[61] and TAMRAD (8·6 months [95% CI 5·9–13·9] *vs* 4·5 months [3·6–8·7]; HR 0·54, 95% CI 0·36–0·81; p<0·01).[62] A statistically significant overall survival benefit was not seen with everolimus in BOLERO-2; median overall survival was 31·0 months (95% CI 28·0–34·6) in the everolimus group versus 26·6 months (22·6–33·1) in the control group (HR 0·89; p=0·14).[63] A similar study, PrE0102,[64] evaluated everolimus in combination with fulvestrant and showed an improved median progression-free survival of 10·3 months (95% CI 7·6–13·8) versus 5·1 months (3·0–8·0 HR 0·61, 95% CI 0·40–0·92; p=0·02). The most common grade 3–4 adverse events in the everolimus group of the BOLERO-2 study were stomatitis (8%), anaemia (6%), dyspnoea (4%), hyperglycaemia (4%), and pneumonitis (3%).[65] Steroid mouth washes to reduce stomatitis incidence have subsequently become a standard of care with everolimus.[66] Starting at a lower dose and escalating dependent on tolerability has also been shown to reduce the side-effects of everolimus while maintaining efficacy.[67] Everolimus is approved irrespective of *PIK3CA* mutation status, and a retrospective analysis of BOLERO-2 participants showed similar efficacy against

tumours with either wild-type or mutant *PIK3CA* (HR 0·43, 95% CI 0·34–0·56 *vs* 0·37, 0·27–0·51).[68]

PI3K–AKT and mTOR inhibitors after CDK4/6 inhibitors

CDK4/6 inhibitors are now the standard of care first-line treatment in combination with endocrine therapy for patients with ER-positive, HER2-negative advanced breast cancer.[69] Multiple trials have shown that progression-free survival in later-line endocrine-based therapies is shorter after CDK4/6 inhibitors.[58,70] Although SOLAR-1 completed enrolment before implementation of CDK4/6 inhibitors as standard first-line treatment, a small subset of patients (n=20 [6%]) had received previous CDK4/6 inhibitors and showed a possible benefit of alpelisib after CDK4/6 inhibition (HR 0·48, 95% CI 0·17–1·36).[54] BYLieve (NCT03056755) is an ongoing phase 2, multicohort, non-comparative trial assessing the benefit of alpelisib after CDK4/6 inhibition; in cohort A (patients must have received a CDK4/6 inhibitor plus an aromatase inhibitor as immediate previous therapy), the median progression-free survival with fulvestrant and alpelisib was 8 months.[71] Subsequent studies have suggested an expected median progression-free survival of 2–3 months for fulvestrant in this population, suggesting alpelisib activity after CDK4/6 inhibition. Interestingly, the duration of previous CDK4/6 inhibition was not associated with progression-free survival on alpelisib and fulvestrant.[72] Notably, the EMA licence for alpelisib is after single-agent hormone therapy, whereas FDA licencing allows previous CDK4/6 inhibitor therapy. The EPIK-B5 trial (NCT05038735) is recruiting and aims to confirm the efficacy and safety of alpelisib plus fulvestrant in a larger population pretreated with a CDK4/6 inhibitor.

In CAPItello-291,[58] subgroup analysis of patients with (n=489) and without (n=219) previous CDK4/6 inhibitor exposure suggested capivasertib activity was unaltered by previous CDK4/6 inhibitor exposure (HR 0·59, 95% CI 0·48–0·72 *vs* 0·64, 0·45–0·90), although absolute progression-free survival was substantially shorter in the CDK4/6 inhibitor-pretreated cohort.[73] Because the original data supporting the use of everolimus pre-dated the approval of CDK4/6 inhibitors, the role of mTOR inhibitors after CDK4/6 inhibitors is unclear. Retrospective, real-world analyses suggest previous exposure to CDK4/6 inhibitors does not substantially affect efficacy.[74,75]

	Patients who received previous CDK4/6 inhibition, n/N (%)*	Efficacy (all)			Efficacy (mutations or alterations in PI3K-AKT pathway)			Efficacy (wild-type PI3K-AKT pathway)		
		Response rate	Median progression-free survival, months	Median overall survival, months	Response rate	Median progression-free survival, months	Median overall survival, months	Response rate	Median progression-free survival, months	Median overall survival, months
Alpelisib										
SOLAR-1[54,55]	20/572 (5·9%)	..	..	..	..	..	..	..	..	..
Fulvestrant + placebo	..	NR	NR	NR	12·8%†	5·7†	31·4†	NR	5·6‡[51]	NR
Fulvestrant + alpelisib	..	NR	NR	NR	26·6%†	11·0†	39·3†	NR	7·4‡[51]	NR
BYLieve[71,78]										
Fulvestrant + alpelisib	127/127 (100%)	NA	NA	NA	19%†	8·0†	27·3†	NA	NA	NA
Capivasertib										
FAKTION[56,57]	0/140	..	..	..	..	..	..	..	..	..
Fulvestrant + placebo	..	8%	4·8	23·4	11%§	4·6¶	20·0¶	13%‖	4·9**	25·2**
Fulvestrant + capivasertib	..	29%	10·3	29·3	47% §	12·8¶	38·9¶	37%‖	7·7**	26·0**
CAPItello-291[58]	489/708 (69·1%)	..	..	..	..	..	..	..	..	..
Fulvestrant + placebo	..	12·2%	3·6	NR	9·7%¶	3·1¶	NR	NR	3·7**	NR
Fulvestrant + capivasertib	..	22·9%	7·2	NR	28·8%¶	7·3¶	NR	NR	5·3**	NR
Everolimus										
BOLERO-2[61,63,65,68]	0/724	..	..	..	..	..	..	..	..	..
Exemestane + placebo	..	0·4%	3·2	26·6	NR	2·96†	NR	NR	2·69‡	NR
Exemestane + everolimus††	..	7·0%	7·8	31·0	NR	7·36†	NR	NR	6·9‡	NR
PrE0102[64]	0/131	..	..	..	..	..	..	..	..	..
Fulvestrant + placebo	..	12·3%	5·1	28·3	NR	NR	NR	NR	NR	NR
Fulvestrant + everolimus‡‡	..	18·2%	10·3	31·4	NR	NR	NR	NR	NR	NR

Sequence of results listed is experimental versus control. NR=not reported. NA=not applicable. *Response rate and median progression-free survival are generally reduced in studies with previous CDK4/6 inhibitor exposure and should be considered when comparing across studies. †Mutated *PIK3CA*. ‡Wild-type *PIK3CA*. §Mutated or altered *PIK3CA* and *PTEN*. ¶Mutated or altered *PIK3CA*, *AKT*, and *PTEN*. ||Wild-type *PIK3CA* and *PTEN*. **Wild-type *PIK3CA*, *AKT*, and *PTEN*. ††With exemestane (everolimus plus exemestane licensed indication). ‡‡With fulvestrant (everolimus with fulvestrant frequently used).

Table 2: **Efficacy in key clinical trials of approved PI3K-AKT and mTOR inhibitors in advanced ER-positive, HER-negative breast cancer**

	Hyperglycaemia		Diarrhoea		Rash*		Stomatitis		Pneumonitis	
	Any grade	Grade 3–4	Any grade	Grade 3–4	Any grade	Grade 3–4	Any grade	Grade 3–4	Any grade	Grade 3–4
Alpelisib										
SOLAR-1[54]	181/284 (63·7%)	104/284 (36·6%)	164/284 (57·7%)	19/284 (6·7%)	153/284 (53·9%)	57/284 (20·1%)	70/282 (24·6%)	7/284 (2·5%)	5/284 (1·8%)	1/284 (<1%)
BYLieve[79]	74/127 (59%)	46/127 (29%)	76/127 (60%)	7/127 (6%)	54/127 (43%)	24/127 (19%)	34/127 (27%)	2/127 (2%)	1/127 (<1%)	0/127
Capivasertib										
FAKTION[56]	29/69 (42%)	3/69 (4%)	56/69 (81%)	10/69 (14%)	36/69 (52%)	14/69 (20%)	10/69 (14%)	0/69	0/69	0/69
Capitello-291[58]	58/355 (16·3%)	8/355 (2·3%)	257/355 (72·4%)	33/355 (9·3%)	135/355 (38%)	43/355 (12·1%)	52/355 (14·6%)	7/355 (2%)	0/355 (0%)	0/355(0%)
Everolimus										
Bolero-2†[65]	13%	4%	30%	12%	36%	1%	56%	8%	12%	3%
PrE0102[64]	12/64 (19%)	2/64 (3%)	25/64(23%)	2/64 (3%)	24/64 (38%)	1/64 (2%)	34/64 (53%)	7/64 (11%)	11/64 (17%)	4/64(6%)

Data are n/N (%). *The group term rash includes the preferred terms of rash, rash macular, maculopapular rash, rash papular, and rash pruritic.†Absolute patient numbers were not available.

Table 3: **Adverse events in key clinical trials of approved PI3K-AKT and mTOR inhibitors in advanced ER-positive, HER2-negative breast cancer**

CDK4/6 inhibitors are increasingly added to adjuvant endocrine therapy in high-risk, ER-positive, HER2-negative breast cancer,[76,77] but the effect of these drugs on response to drugs targeting PI3-AKT or mTOR in the advanced or metastatic setting is unknown. The efficacy and toxicity of the approved pathway inhibitors are compared in tables 2 and 3.

Precision medicine and predictive biomarkers

Despite substantial research efforts, only mutations or alterations that activate the PI3K-AKT pathway have been approved by the FDA as predictive biomarkers. *PIK3CA* mutations predict efficacy of α-selective PI3K inhibitors but not of the mTOR inhibitor everolimus,[80] possibly reflecting multiple inputs into mTOR activity other than PI3K-AKT pathway signalling.

SOLAR-1[54] recruited patients with selected *PIK3CA* mutations, including 11-codon substitutions in exons 7, 9, and 20. Real-world data in patients with other rare activating *PIK3CA* mutations, not eligible for SOLAR-1, provide some support for alpelisib activity[81] but suggest it might be lower with rare *PIK3CA* 1258T>C (Cys420Arg) mutations, which accounted for only 2% of patients in SOLAR-1.[82] Strong preclinical data suggest that cancers with double *PIK3CA* mutations are particularly sensitive to PI3K inhibition, and a small amount of clinical data suggests that patients with double *PIK3CA* mutations have higher response rates to taselisib[26] and longer progression-free survival on alpelisib.[83] The presence of a double *PIK3CA* mutation in a tumour is therefore a moderately strong selection factor for considering alpelisib therapy versus alternative therapies.

Capivasertib is more active in PI3K-AKT pathway-altered cancers (ie, with specific *PIK3CA, AKT1,* or *PTEN* alterations) than in cancers without such alterations. In CAPItello-291, HRs for progression-free survival were 0·50 (95% CI 0·38–0·65; p<0.001) in patients with alterations and 0·79 (0·61–1·02) in those without.[58] For overall survival, HRs in FAKTION were 0·46 (0·27–0·79; p<0.0047) and 0·86 (0·49–1·52; p=0.60),[57] with PI3K-AKT pathway alteration a predictive biomarker. The sensitivity for detecting homozygous deletions of *PTEN* is low in ctDNA analysis, supporting the preference to test using tissue samples. Conversely, ctDNA analysis after progression on CDK4/6 inhibitors can detect acquired pathway alterations that are not present in an archival tissue sample. These findings suggest that combined tissue and ctDNA analysis to robustly identify pathway alterations might become the standard now that capivasertib is licensed.

Resistance and other ongoing challenges

The clinical benefit of PI3K-AKT and mTOR inhibitors, like that of most targeted cancer drugs, is limited by intrinsic and acquired resistance. Documented mechanisms of PI3K inhibitor resistance include reactivation of the signalling pathway and activation of compensatory parallel signalling cascades,[84] promoted by physiological feedback loops. For example, inhibition of PI3K-AKT induces expression of RTKs and RTK adaptors, such as GRB10 and IRS, and suppression of PTEN translation.[85,86] Moreover, these signals can induce key parallel pathways such as oestrogen receptor signalling.

In some cases, acquired drug resistance to p110α-specific inhibitors can be mediated by loss of PTEN, which, in turn, leads to increased signalling through the PI3K p110β isoform.[36,87] Analyses of plasma and tumour samples from the phase 1 trial (NCT01870505) of alpelisib identified loss-of-function *PTEN* mutations in 25% of patients with de-novo resistance.[88] Everolimus inhibits the mTORC1 complex, initiating a negative feedback loop that induces activation of mTORC2 and AKT and potentially causes treatment resistance.[89] Vistusertib, a dual inhibitor of mTORC1 and mTORC2, showed high activity in preclinical breast cancer models through more potent pathway inhibition. Unfortunately, when tested in the MANTA trial, vistusertib was less effective than everolimus on a

fulvestrant backbone; median progression-free survival was 8·0 months (95% CI 5·6–9·9) with fulestrant plus vistusertib versus 12·3 months (7·7–15·7) with fulvestrant plus everolimus (HR 0·63, 95% CI 0·45–0·90; p=0·01).[90] These conflicting results probably reflect dosing in the clinical setting being limited by the adverse effects of vistusertib, ultimately resulting in less potent mTORC1 inhibition compared with everolimus. mTOR inhibition upregulates RTKs, including IGFR-1, although combined inhibition of IGFR-1 and IGFR-2 with mTOR was ineffective in a randomised phase 2 trial, suggesting that IGFRs might not be the main upregulated RTKs.[91]

Drug toxicity and tolerability are the major challenges with inhibitors of the PI3K-AKT and mTOR pathways. In SOLAR-1, the toxicity of alpelisib remained an issue, with 71 (25%) of 284 patients discontinuing treatment,[54] but real-world analyses suggest that, in clinical practice, toxicity and discontinuation rates might be higher.[92] In SOLAR-1, hyperglycaemia was more pronounced in patients who were diabetic or prediabetic at baseline.[93] In CAPitello-291, 46 (13%) of 355 patients discontinued due to adverse events;[59] the more manageable toxicity profile of capivasertib, with a low hyperglycaemia incidence, might be partly due to the intermittent dosing schedule (ie, twice per day for 4 days followed by 3 days off). Rigorous safety monitoring of these side-effects in clinical practice is paramount, with prompt action to prevent escalation to high-grade events.

Perspectives on selective strategies

The first-line treatment for most patients with ER-positive metastatic breast cancer is endocrine therapy plus one of three approved CDK4/6 inhibitors, irrespective of *PIK3CA*, *AKT1*, or *PTEN* mutation status. No studies have investigated replacing CDK4/6 inhibitors with PI3K-AKT pathway inhibitors in the first-line setting; this change has been considered inappropriate, given the overall survival benefit and comparatively better safety of CDK4/6 inhibitors.

In the second-line setting, multiple approved options depend upon *BRCA1* or *BRCA2* mutations, PI3K-AKT pathway alterations, and *ESR1* status. Before the approval of capivasertib, alpelisib was generally considered ahead of everolimus for patients with *PIK3CA*-mutant cancers, influenced by the substantially improved response rates with alpelisib, lower rates of pneumonitis and stomatitis, and the concept of precision medicine.

Alpelisib is approved only for the *PIK3CA*-mutant population, whereas the recent approval criteria for capivasertib includes tumours with specific alterations in *PIK3CA*, *AKT1*, or *PTEN*. The main decision for clinicians is the strategic use of PI3K and AKT inhibitors for a given patient in clinic. For patients with *AKT1* and *PTEN* alterations, the decision to use capivasertib is straightforward. For patients with *PIK3CA* mutations, there is a more complex choice between the use of alpelisib and capivasertib. In FAKTION, capivasertib showed a substantial overall survival benefit in the pathway-altered population,[57] whereas for alpelisib in SOLAR-1, a non-significant increase in overall survival was observed[55] (table 2). Cross-trial comparison between CAPItello-291 and SOLAR-1 is particularly difficult due to the differences in the patient populations, in particular previous CDK4/6 inhibitor use, which, as previously noted, is associated with substantially shorter subsequent progression-free survival in the context of later-line endocrine-based therapy. Considering these factors, alpelisib and capivasertib seem to have similar activity with respect to progression-free survival, with overall survival comparisons awaiting data from CAPItello-291. Additionally, although AKT is the dominant signalling node downstream of PI3K, PI3K signalling can also occur independent of AKT.[94] As such, PI3K inhibition might theoretically be superior to AKT inhibition. Conversely, PTEN loss is an acquired mechanism of resistance to alpelisib but not capivasertib.

Safety is a key feature affecting successful targeting of the PI3K-AKT and mTOR pathways. As seen with vistusertib (dual inhibitor of mTORC1 and mTORC2), a theoretically better drug can be less effective in the clinic if it is associated with more side-effects. From this perspective, the adverse event profile of capivasertib is generally more favourable than that of alpelisib. Although CAPItello-291 included a much broader population of patients (eg, with impaired glucose tolerance and tablet-controlled diabetes) than SOLAR-1, hyperglycaemia was substantially more common with alpelisib, and rash and stomatitis were slightly more common; in contrast, diarrhoea was more common with capivasertib (table 3). The choice of drug might depend on both an individual's comorbidities and the relative importance of these toxicities for individual patients. Capivasertib is a treatment option for a larger population of patients with glucose tolerance issues. Alpelisib might have higher rates of adverse effects in Asian individuals.[95,96]

The BELLE-3 study showed that previous everolimus did not affect the efficacy of the PI3K inhibitor buparlisib;[50] thus, everolimus could have activity after alpelisib and vice versa, although aside from this study, there are few data available. There are no data on cross-resistance between capivasertib and mTOR inhibitors as patients who had previously received mTOR inhibitors were excluded from CAPitello-291.

ESR1 mutations should be considered in the selection strategy for sequencing therapies because they occur in up to 40% of patients who have received an aromatase inhibitor.[97] *ESR1* mutations are potentially sensitive to selective oestrogen receptor degraders (SERDs). The first oral SERD, elacestrant, was approved by the FDA in January, 2023, for patients with ER-positive, HER2-negative, *ESR1*-mutated advanced breast cancer on the basis of progression-free survival results from the phase 3

EMERALD study.[70] Camizestrant is a next-generation oral SERD that has shown significantly improved progression-free survival over fulvestrant in the phase 2 SERENA-2 study,[98] with multiple ongoing phase 3 studies awaited.

Adjuvant and neoadjuvant therapy

Although everolimus has shown benefit as an adjuvant therapy in resected renal cell carcinoma, adjuvant studies in breast cancer have shown no benefit. The UNIRAD trial[99] evaluated everolimus plus hormone therapy in patients with high-risk, early stage, ER-positive breast cancer and showed no benefit in invasive disease-free survival (HR 0·95, 95% CI 0·69–1·32; p=0·77) or overall survival (HR 1·09, 95% CI 0·62–1·92; p=0·75).[99] The phase 3 SWOG S1207 trial, which evaluated everolimus in the same clinical setting, also did not show improved outcomes in terms of invasive disease-free survival (HR 0·93, 95% CI 0·76–1·14) and overall survival (HR 0·98, 95% CI 0·75–1·28).[100] There are various potential explanations for these negative results. For example, the studies were conducted before the prophylactic use of steroid mouthwashes, leading to high incidence of stomatitis (any grade in 417 [66%] of 625), with 319 (53%) of 625 patients in the everolimus group of UNIRAD discontinuing due to side-effects or personal decision.[99] In BOLERO-2,[61] the majority of benefit was improved progression-free survival, with only a minor improvement in response rates, suggesting that adjuvant everolimus might have resulted in stability as opposed to eradication of micrometastatic disease. Finally, mTOR signalling is activated as a mechanism of resistance to endocrine therapy and might not be important before the development of resistance in the adjuvant setting.

The NEO-ORB study investigated the addition of neoadjuvant alpelisib to endocrine therapy in post-menopausal women with ER-positive, HER2-negative early breast cancer, with no improvement in overall response rate in either the *PIK3CA*-mutant or wild-type cohort and low pathological complete response rates in all groups.[101] Only 68 (52%) of 131 patients in the alpelisib group completed the full 24 weeks of neoadjuvant alpelisib. These generally disappointing results indicate that activation of PI3K signalling in advanced endocrine-resistant cancer might be important for the efficacy of alpelisib and potentially other PI3K-AKT pathway inhibitors.[102]

Future directions

Triplet therapy

Inavolisib is an α-selective PI3K-specific inhibitor that also promotes degradation of mutant p110α, potentially limiting the consequences of feedback upregulation of RTKs.[103] Preclinical models have shown synergy between CDK4/6 inhibitors and PI3K inhibitors, with PI3K inhibitors blocking the development of CDK4/6 inhibitor resistance.[104]

The INAVO120 phase 3 trial compared inavolisib plus palbociclib and fulvestrant with placebo in first-line therapy of patients with ER-positive, HER2-negative, *PIK3CA*-mutant advanced breast cancer who recurred on or within 12 months of adjuvant endocrine therapy.[105] The study was enriched for patients with poorer prognosis, with 260 (80%) of 325 patients having visceral disease and 168 (50%) having liver metastases. The addition of inavolisib more than doubled median progression-free survival compared with placebo (15·0 months [95% CI 11·3–20·5] *vs* 7·3 months [5·6–9·3]; HR 0·43, 95% CI 0·32–0·59; p<0·0001), with a non-significant difference in overall survival at the first interim analysis (HR 0·64, 95% CI 0·43–0·97, p=0·0338) (Jhaveri KL, Memorial Sloan Kettering Cancer Center, personal communication). The toxicity profile of inavolisib was manageable, with a discontinuation rate of 6·8%. Most notably, 51% of patients experienced any-grade stomatitis (≥grade 3 in 6%), which, going forward, will require prophylactic management. Inavolisib had not received regulatory approval at the time of writing, although in the future, triplet first-line therapy might become a standard for patients with more aggressive cancers. CAPItello-292 (NCT04862663) is a phase 3 study evaluating the safety and efficacy of capivasertib in triplet combination with the CDK/6 inhibitor palbociclib and fulvestrant in CDK/6 inhibitor-naive patients.

Anticipated phase 2 and 3 results

Anticipated results from phase 2 and phase 3 trials include those from FAIM (NCT04920708), which is evaluating ipatasertib (in combination with palbociclib and fulvestrant), and FINER (NCT04650581), which is evaluating ipatasertib in combination with fulvestrant in patients who have received previous CDK/6 inhibition. Capivasertib is also being investigated in triple-negative breast cancer (NCT03997123) and prostate cancer (NCT04493853). INAVO121 is a phase 3 trial that is also evaluating the combination of fulvestrant with inavolisib in the second-line setting after CDK/6 inhibition (NCT05646862). Ongoing trials of novel PI3K-AKT and mTOR inhibitors are reported in table 1.

Novel mutant-specific inhibitors

The efficacy of PI3K inhibitors in *PIK3CA*-mutant cancers is thought to be primarily driven by the inhibition of mutant *PI3K*, but the dose and clinical activity of the approved inhibitor alpelisib is limited by toxicity from the non-selective inhibition of wild-type *PI3K*α. As such, selective inhibition of mutant *PI3K*α might result in improved therapeutic index and reduced off-tumour toxicity. LOXO-783, a potent, mutant-selective allosteric PI3Kα-His1047Arg inhibitor, induced additive effects on tumour regression in ER-positive, HER2-negative, *PI3K*α 3140A>G (His1047Arg) breast cancer models and is now being evaluated in the phase I trial PIKASSO-01 (NCT053077050).[106] The allosteric, pan-mutant, selective PI3Kα inhibitor RLY-2608 is being evaluated in combination with fulvestrant in the phase 1 ReDiscover

trial (NCT05216432), and, to date, has demonstrated target inhibition and a favourable toxicity profile, with no documented grade 3 hyperglycaemic events.[107]

Dual inhibitors

Preclinical studies have shown that inhibitors of PI3K-AKT and mTOR induce signalling feedback loops that bypass the effects of targeted blockade and limit their anti-tumour effects.[103] These findings have led to research into vertical inhibition of this pathway through dual inhibitors to enhance efficacy. Preclinically, vertical pathway inhibition with dual PI3K and mTOR inhibitors is highly effective at limiting negative feedback. However, continuous dual inhibition of PI3K and mTOR is not sufficiently safe to be an effective clinical strategy. Gedatolisib is a potent intravenous dual inhibitor that selectively targets all class I isoforms of PI3K and mTOR, with promising antitumour activity and a manageable toxicity profile in phase 1 trials. VIKTORIA-1, the phase 3 gedatolisib trial (NCT05501886) will investigate whether highly potent intermittent pathway inhibition, achievable through intermittent intravenous therapy, is more effective than continuous, less potent pathway inhibition, achievable through continuous oral therapy. Capivasertib also reportedly inhibits S6K in preclinical studies,[108] potentially contributing to vertical pathway inhibition.

ctDNA-directed therapy

Finally, early changes in ctDNA levels in patients with metastatic breast cancer could permit early prediction of response to therapy, with rapid decreases in tumours that respond to therapy and minor or no decreases in tumours that do not respond. Early changes in ctDNA are predictive of progression-free survival in patients with advanced breast cancer, based on findings from the phase 3 PALOMA trial, in which patients without ctDNA suppression after 2 weeks of palbociclib and fulvestrant had significantly shorter progression-free survival than those with ctDNA suppression.[109] The FAIM phase 2 open-label trial (NCT04920708) is recruiting patients with advanced ER-positive, HER2-negative breast cancer on first-line fulvestrant and CDK/6 inhibitor to ctDNA dynamic monitoring, randomly assigning patients without ctDNA suppression to fulvestrant plus palbociclib with or without ipatasertib. This trial might allow escalation to triplet therapy in patients predicted to have poor response to doublet therapy, as an alternative strategy to INAVO120 and CAPItello-292. SAFIR 03 (NCT05625087) uses ctDNA dynamics to randomise patients to either to ribociclib or alpelisib in combination with fulvestrant.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms (alone and in combination) "breast cancer", "PI3K pathway", "targeting PI3K", "targeting AKT", "targeting mTOR", and "PI3k inhibitor resistance". No date restrictions were applied to this search, and the search was done between June 23, 2023, and Dec 23, 2023. Only papers published in English were reviewed. The final reference list was generated on the basis of originality, impact, and relevance to the scope of this Review.

Conclusions

Despite the huge advances made to date in targeting the PI3K-AKT and mTOR pathways, major challenges remain. Issues arise from both the toxicity profiles of these drugs (due to the essential nature of PI3K-AKT in cellular function) and the induction of negative feedback loops that oppose pathway inhibition. Continuing to develop inhibitors that reduce normal tissue toxicity and mitigate feedback remains the biggest challenge for drug development. With three inhibitors in the pathway licenced, the key clinical issue is the appropriate sequencing of these drugs, as well as other therapies, such as new oral SERDs and chemotherapies, including antibody–drug conjugates. Another clinical challenge is the choice of inhibitor for *PIK3CA*-mutant breast cancer, with the adverse event profile the clearest indicator of difference between alpelisib and capivasertib. Expanding evidence suggests pathway alterations might be acquired through endocrine therapy and CDK/6 inhibitor use, with precision medicine increasingly suggesting that broad analysis of both tumour biopsy and progression ctDNA might be required to extensively identify pathway alterations. Such serial and comprehensive analysis could identify more patients who might benefit from these interventions.

Future challenges include the further development of drugs with a higher therapeutic index, for which mutant-selective PI3Kα inhibitors hold promise, the development of combinations with oral SERDs, establishment of predictive biomarkers, and identification of patients requiring early escalated therapy in combination with CDK/6 inhibitors. We hope that ongoing prospective trials will answer these questions and enable the optimal sequencing of these targeted drugs for the correct patient populations, with manageable toxicity profiles and at the most appropriate time in their cancer journey.

Contributors

IMB and NCT prepared the original manuscript, including illustrations, and were involved in the editing of the final manuscript. FA, LAC, and SC were involved in the critical revision of the manuscript. All authors approved the version to be published.

Declaration of interests

NCT has received advisory board honoraria from AstraZeneca, Lilly, Pfizer, Roche–Genentech, Novartis, GlaxoSmithKline, Repare Therapeutics, Relay therapeutics, Zentalis, Gilead, Inivata, Guardant, and Exact Sciences; and research funding from AstraZeneca, Pfizer, Roche–Genentech, Merck Sharpe & Dohme, Guardant Health, Invitae, Inivata, Personalis, and Natera. FA has received consulting fees from MEDIMMUNE, Gilead, Relay Therapeutics, Guardant Health, and Lilly; and institutional research grants from AstraZeneca, Daiichi Sankyo,

Roche, Lilly, Pfizer, Owkin, Novartis, and Guardant Health. SC has received grants from Daiichi Sankyo, AstraZeneca, Paige.ai, Sanofi, Genentech, Novartis, and AmbrX; and consulting fees from Novartis, Lilly, AstraZeneca, Nuvalent, Boxer Capita, Gerson Lehman Group, Genesis Therapeutics, Sanofi, Effector Theapeutics, Neogenomics, SAGA Diagnostics, and Prelude Therapeutics. SC holds non-financial board roles with Odyssey Biosciences and the Breast Cancer Research Foundation, has stock options with Effector Therapeutics and Totus Medicines, and holds patents for CDK4 and CDK6 proteolysis targeting chimeras. IMB and LAC declare no competing interests.

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