The PI3K Pathway: Background and Treatment Approaches

Michael P. Lux   Peter A. Fasching   Michael G. Schrauder   Alexander Hein   Sebastian M. Jud
Claudia Rauh   Matthias W. Beckmann

University Breast Center for Franconia, Department of Gynecology, Erlangen University Hospital, CCC Erlangen-EMN, Erlangen, Germany

Summary

Two-thirds of all breast cancer patients with metastases have a hormone receptor (HR)-positive/human epidermal growth factor receptor 2 (HER2)-negative subtype. Endocrine therapy is the treatment of choice in these patients since in addition to its effectiveness it can also maintain the patients' quality of life over a longer term. However, 44–62% of postmenopausal patients with metastatic breast carcinoma have primary tamoxifen resistance. After 3–5 years, 30–40% of the patients receiving tamoxifen treatment develop secondary resistance. Understanding the way in which resistance develops is therefore essential for developing treatment approaches that can prevent or reverse endocrine resistance. The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway plays a central role here. As a result of the numerous interactions involved, complex issues arise that need to be taken into account in the development and use of therapeutic agents. In addition, this signaling pathway is the one that most frequently undergoes mutations in breast cancer. The prognostic and predictive significance of individual mutations has not yet been fully explained, but it might provide a basis for patient selection in clinical studies. Initial research results on the use of PI3K inhibitors suggest that this may be a highly promising therapeutic approach, with an acceptable side effect profile.

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Introduction

Although the prognosis in patients with the luminal A/B subtype is basically better than in those with triple-negative or human epidermal growth factor receptor 2 (HER2)-positive breast carcinoma, patients with hormone receptor (HR)-positive/HER2-negative breast cancer represent two-thirds of all breast cancer patients with metastases [1]. Endocrine therapy is the first-line treatment option for metastatic HR-positive breast carcinoma [2]. In general, endocrine therapy is preferable since, in addition to being effective, it also maintains the patient's quality of life, in comparison with chemotherapy.

Endocrine resistance is a major problem, and a distinction is made between primary and secondary resistance. A significant proportion of patients have de novo or primary resistance, or they develop endocrine resistance during the course of treatment. Only 50% of the patients with metastatic estrogen receptor (ER)-positive breast cancer benefit from first-line endocrine therapy, and all of those who respond initially acquire resistance, with tumor relapse and growth [3]. With secondary resistance as well, the progression-free survival (PFS) times with additional endocrine treatment options are short.

Therefore, major goals are to understand the way in which endocrine resistance arises and to develop therapeutic agents that can reverse it or prevent it from developing. In this context, the focus is on the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway. This is the most frequently mutated signaling pathway in breast carcinoma. Both experimental and clinical data support the hypothesis that, in addition to mitogen-activated protein kinase (MAPK/ERK), the pathway plays a significant role in endocrine resistance.

The PI3K/Akt/mTOR Signaling Pathway

PI3K/Akt/mTOR is an intracellular signaling pathway that responds to hormones and growth factors. Its activation leads to nu-

Fig. 1. Overview of the PI3K/Akt/mTOR pathway (reproduced from [9], with permission). RTK = receptor tyrosine kinase, P = phosphate, IRS1 = insulin receptor substrate-1, PIP_2 = phosphatidylinositol 4,5-bisphosphate, PIP_3 = phosphatidylinositol 3,4,5-trisphosphate, PI3K = phosphatidylinositol 3-kinase, Akt = activated protein kinase B, mTOR = mammalian target of rapamycin, rictor = rapamycin-insensitive companion of mTOR, raptor = regulatory-associated protein of mTOR, TSC = tuberous sclerosis complex, Rheb = Ras homolog enriched in brain, GDP = guanosine diphosphate, GTP = guanosine triphosphate, S6K1 = ribosomal S6 kinase 1, 4EBP1 = 4E-binding protein 1, eIF4e = eukaryotic translation initiation factor 4E.
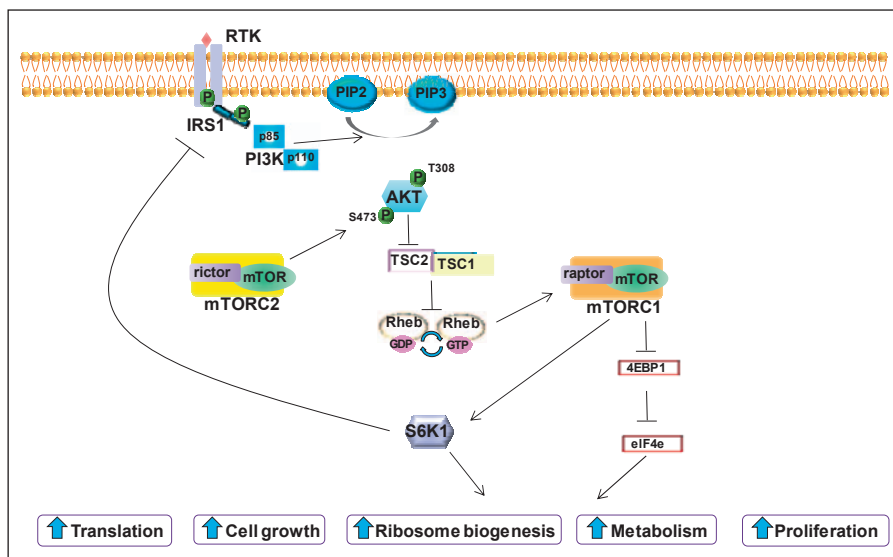
merous cellular processes such as increased cell growth, cell proliferation, and cellular motility, a shift toward glycolytic metabolism, and also increased cell migration and deregulated apoptosis [4].

PI3Ks are grouped into 3 classes (I–III) based on their structure and substrate specificity [5]. The PI3K heterodimer belonging to class IA plays a central role in the signaling pathway [6]. The heterodimer consists of 2 subunits. The regulatory subunit, p85, controls the activation of the catalytic subunit, p110, depending on the stimulation of growth receptor tyrosine kinases [7]. Subunits p110α, p110β, and p110δ are encoded by *PIK3CA*, *PIK3CB*, and *PIK3CD*, while the regulatory subunit is encoded by *PIK3R1*, *PIK3R2*, and *PIK3R3* [8]. p110α and p110β are ubiquitously expressed. Class IB consists of p110γ, which is encoded by *PIK3CG*. Expression of p110δ and p110γ is generally limited to hematopoietic and immunological cells.

Activation takes place through the binding of various extracellular growth factors (e.g., epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), and insulin) to their membrane-bound tyrosine kinase receptors (fig. 1) [9]. The respective receptor dimerizes, is autophosphorylated and recruits adaptor proteins such as insulin receptor substrate (IRS)1 and IRS2. Subsequent phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP_2) into phosphatidylinositol-3,4,4-trisphosphate (PIP_3) leads to the phosphorylation of Akt, a serine/threonine kinase that further influences the tumor cell cycle for growth and survival (fig. 1) [10].

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is an important tumor suppressor that has a contrary effect and dephosphorylates PIP_3 to PIP_2. PIP_3 levels are thus directly regulated by the opposing effects of PI3K and PTEN.

mTOR is a serine/threonine kinase that is downstream from PI3K and Akt. Two different complexes involving mTOR, mTORC1 and mTORC2, have different functions. mTORC1 is activated by inhibition of tumor suppressor tuberous sclerosis 1/2 (TSC1/2) and subsequently influences the cell metabolism, leading to anabolic cell growth.

Activation of mTORC1 also leads to a negative feedback loop [9]. Direct inhibition of mTORC1 by an administered treatment can suspend the negative feedback loop, leading to the paradoxical activation of the signaling pathway and expression of multiple receptor tyrosine kinases such as HER2 and the IGF-1 receptor (IGF-1R) [11]. Both this paradoxical activation and the activation of alternative signaling pathways need to be taken into account in the context of therapeutic approaches.

The PI3K Signaling Pathway and Endocrine Resistance

Deregulation of the class 1A PI3K signaling pathway is associated with the development of resistance to numerous forms of treatment, including antiestrogens, trastuzumab, radiotherapy, and chemotherapy [12]. It has been demonstrated experimentally that PI3K activation is associated with both de novo and also acquired endocrine resistance.

Preclinical data have confirmed that there is crosstalk between the ER and the PI3K/Akt/mTOR signaling pathway. Studies using breast carcinoma cell lines have shown an association between the activity of the Akt signaling pathway and endocrine resistance. Subsequently, an association was demonstrated between the activation of Akt or reduced PTEN expression and endocrine resistance in patients with metastatic breast carcinoma or recurrences after tamoxifen therapy [13, 14]. Inhibition of Akt via the PI3K signaling pathway may in turn restore sensitivity to tamoxifen, aromatase inhibitors, and also fulvestrant [9].

Activation of the PI3K signaling pathway can be measured using the phosphorylated secondary products. A gene expression signature for PI3K activation based on the levels of phosphoprotein markers (P-AKT, P-p70S6K) is increased in HR-positive carcinomas, particularly in luminal B breast carcinomas. This suggests that luminal B tumors have higher levels of PI3K activity that may lead to a reduced response to antiestrogen therapy [3, 15].

Table 1. Alterations in the PI3K signaling pathway (adapted from [12, 16])

Gene	Aberration	Effect on signaling	Frequency			Prognosis
			HR-positive/ luminal	HER2-positive	TNBC/ basal-like	
ERB2	gene amplification or overexpression	hyperactivation of Erb2 signaling (PI3K, MEK)	10	100	0	correlates with poorer outcome
PTEN	loss of function/ reduced expression	hyperactivation of PI3K signaling	29–44	22	67	no consistent correlation
PIK3CA	activating mutation	hyperactivation of PI3K signaling	28–47	23–33	8	ER+/HER2–, better outcome; ER+/HER2+, poorer outcome
PIK3CB	amplification	unknown				unknown
IGF1R, INSR	receptor activation	activates IGF-1R/InsR signaling (PI3K, MEK)	41–48	18–64	42	unknown
FGFR1	amplification	hyperactivation of FGFR signaling (PI3K, MEK)	8.6–11.6	5.4	5.6	correlates with shorter RFS
INPP4B	reduced expression or genomic loss	hyperactivation of PI3K signaling	8	38	88	correlates with poorer outcome
AKT1	activating mutation	hyperactivation of Akt signaling	2.6–3.8	0	0	unknown
AKT2	amplification	hyperactivation of Akt signaling	2.8			unknown

EGFR = Epidermal growth factor receptor, ER = estrogen receptor, FGFR = fibroblast growth factor receptor, HER2 = human epidermal growth factor receptor 2, HR = hormone receptor, IGF-1R = insulin-like growth factor-1 receptor, InsR = insulin receptor, MEK = MAPK/ERK kinase, mTORC1 = mammalian target of rapamycin complex 1, PI3K = phosphatidylinositol 3-kinase, RFS = relapse-free survival, TNBC = triple-negative breast cancer.

Mutations in the PI3K Signaling Pathway and Their Prognostic and Predictive Values

An overview of aberrations in the PI3K signaling pathway is presented in table 1. Loss of the *PTEN* gene and mutations in the *PIK3CA* gene are some of the most frequent aberrations seen in malignancies such as breast cancer [6]. It is estimated that the PI3K/Akt/mTOR signaling pathway is activated in 70% of all breast carcinomas as a result of genetic alterations [16].

PIK3CA

The highest frequency of mutations in *PIK3CA*, which codes for the catalytic subunit p110α, is found in ER-positive tumors and particularly in the luminal A subtype (luminal A, 45%; luminal B, 29%) [6]. A meta-analysis including 26 studies demonstrated a significant association between *PIK3CA* mutations and ER and progesterone receptor (PR) expression (odds ratio (OR) 1.92, 95% confidence interval (CI) 1.65–2.23; OR 1.88, 95% CI 1.61–2.20, respectively) [17]. Although these mutations often occur in ER-positive breast carcinomas, their clinical relevance and their prognostic and predictive value are still matters of debate.

Up to 80% of the *PIK3CA* mutations occur in hotspots in the helical (E542K and E545K in exon 9) and kinase (H1047R or H1047L in exon 20) domains of p110α [12]. Exon 9 mutations are considered to enable p110α to escape the inhibitory effect of p85 via the Src homology 2 (SH2) domain [5]. These mutations increase the PI3K activity and induce cellular transformation in vitro and tumorigenicity in vivo when overexpressed in human mammary epithelial cells. They also induce mammary tumor formation in transgenic mice [10, 12, 18]. However, *PIK3CA* mutations have not been found to be associated with increased phosphorylation of Akt, S6, or 4EBP1, which actually represent markers for an activated PI3K signaling pathway. Overall, the data are still inconsistent and might also be explained by a lack of standardization in the measurement of phosphorylated proteins. Moreover, the acquisition of mutations in *PIK3CA* does not appear to be responsible for the development of endocrine resistance. Arthur et al. [19] have profiled matched primary and recurrent samples from 120 breast cancer patients treated with endocrine therapy. 43% of the patients had at least 1 *PIK3CA* mutation at diagnosis and 41% at the time of recurrence. Only 8% of the patients changed their *PIK3CA* mutation status (4 gains, 2 losses) with local recurrence, metastatic disease, or progression on primary endocrine treatment.

In retrospective studies, *PIK3CA* mutations have been found to be associated with a good long-term outcome in primary ER-positive tumors [20]. A meta-analysis including 5 studies noted a significant correlation between *PIK3CA* mutations and a better recurrence-free survival (hazard ratio (HR) 0.76, 95% CI 0.59–0.98; P = 0.03) [17]. Another meta-analysis including 6 studies found a similar result in relation to disease-free survival (HR 0.72, 95% CI 0.57–0.91; P = 0.006) [21]. A further study analyzed the association between *PIK3CA* mutations and outcome in 4,540 postmenopausal patients with ER-positive breast cancer who received endocrine therapy in the context of the TEAM trial, a multinational, randomized phase III trial comparing exemestane with tamoxifen for

the first 2.5–3 years, followed by exemestane [22]. *PIK3CA* mutations were frequent (at 39.8%) and most of the mutations were detected in the sequences coding for the helical or kinase domains (E542K 13.5%, E545K 21.9%, H1047R 44.3%). Patients with *PIK3CA* mutations were at significantly lower risk of distant metastases after 5 years (distant relapse-free survival 91% vs. 88%, HR 0.76, 95% CI 0.63–0.91; P = 0.003), but the multivariate analysis failed to show that the *PIK3CA* mutation status was an independent prognostic marker. The authors also examined the effects of mutations in the helical versus kinase domains. The influence of these different mutations on the outcome is still a matter of controversy. No statistically significant differences were observed between helical and kinase domain mutations in the study (HR 0.82, 95% CI 0.60–1.14; P = 0.24).

Overall, *PIK3CA* mutation and increased ER positivity can thus be regarded as favorable prognostic parameters. However, these considerations are mainly related to ER-positive and HER2-negative carcinomas in the context of endocrine therapy. Several publications have shown that, in HER2-positive breast carcinomas, the *PI3KCA* mutation represents a negative factor for the outcome of treatment with trastuzumab. A retrospective study with tumor samples from 240 patients with HER2-positive early breast carcinoma who were treated with epirubicin, 5-fluorouracil, and cyclophosphamide followed by 1 year of trastuzumab noted reduced overall survival in the presence of the mutation in comparison with the wild type (multivariate HR 2.14, 95% CI 1.01–4.51; P = 0.046) [23]. In relation to the metastatic situation, a retrospective study including 139 patients with HER2-positive tumors and treatment including trastuzumab found that *PTEN* loss and *PIK3CA* mutation represented significant independent parameters associated with a shorter time to progression and reduced survival [24]. However, it is still questionable whether the *PIK3CA* mutation represents a purely prognostic factor in HER2-positive tumors or whether it may also be predictive for anti-HER2 therapy, since most retrospective studies have not included a control arm without anti-HER2 therapy. In the framework of the FinHER study, in which patients received either trastuzumab or chemotherapy alone for 9 weeks, the *PIK3CA* mutation status was not found to be predictive of a benefit from trastuzumab [25].

In summary, it may be noted that *PIK3CA* mutations:
- occur most often in ER-positive and HER2-negative tumors;
- may coexist with additional PI3K-activating mechanisms, such as *HER2* amplification or *PTEN* loss;
- may potentially represent a favorable prognostic parameter in the luminal subtypes; and
- may be a predictive factor for a poorer outcome under trastuzumab-based therapies [5].

PTEN

PTEN is a tumor suppressor that deactivates the PI3K signaling pathway. *PTEN* loss often coexists with *PIK3CA* mutations [5]. Switching-off of *PTEN* and also overexpression of oncogenes that activate the PI3K/Akt signaling pathway are associated with endocrine resistance to tamoxifen and fulvestrant [26]. Loss of *PTEN* gene expression in HR-positive carcinomas is also predictive of reduced recurrence-free survival after tamoxifen [27]. However, re-

Table 2. Overview of PI3K inhibitors

Class	Substance
Pan-PI3K inhibitors	BKM120 (buparlisib)
	XL-147
	PX-866
	PKI-587
	GDC-0941
p110α-specific inhibitors	wortmannin
	LY294002
	BYL719 (alpelisib)
	GDC-0032
	INK-1117
p110δ-specific inhibitors	CAL-101
Dual PI3K/mTOR inhibitors	BEZ235
	BGT226
	PF-4691502
	GDC-0980
	XL-765

duced *PTEN* expression is more often observed in triple-negative breast carcinomas (67% vs. 29–44% in ER-positive tumors) [28]. Tumor cells with *PTEN* loss tend to depend more on *PIK3CB* than on *PIK3CA*. A pan-PI3K inhibitor might be considered preferable here over a selective p110 inhibitor.

INPP4B

Inositol polyphosphate 4-phosphatase type IIB (INPP4B) is another potential biomarker. It is a tumor suppressor that regulates PI3K/Akt and is involved in the dephosphorylation of PIP_3 to PIP_2. Its deletion correlates with poor prognosis. Patients with an ER-positive/INPP4B-deficient tumor show reduced survival in comparison with patients with an ER-positive/INPP4B-positive tumor [29]. *INPP4B* deficiency might be a candidate for the application of PI3K inhibitors [6].

Therapeutic Approaches

The importance of the PI3K signaling pathway in relation to endocrine resistance and also the high frequency of alterations in the pathway in breast carcinoma make it an interesting therapeutic target. Multiple inhibitors of the PI3K/Akt/mTOR signaling pathway are currently in the process of preclinical development or have already been investigated in clinical studies. At present, more than 100 studies listed on ClinicalTrial.gov investigate the use of PI3K inhibitors in breast carcinoma. A distinction is made between pan-PI3K inhibitors, p110α-specific inhibitors, p110δ-specific inhibitors, and dual PI3K/mTOR inhibitors (table 2). It is as yet unclear whether pan-PI3K inhibitors or isoform-selective inhibitors represent the more effective approach.

As the ER persists in most ER-positive breast carcinomas with resistance to endocrine therapy and a response to estrogens is possible, PI3K inhibitors are combined with an antiestrogen therapy. Somatic aberrations such as *PIK3CA* and *AKT1* mutations, *PTEN* and *INPP4B* loss, and *PIK3CB* and *AKT2* amplification may indi-

cate either disturbed activation or dependency on the PI3K signaling pathway. On the basis of the existing data, it is assumed that *PIK3CA* mutations and *PTEN* loss increase the sensitivity to PI3K/Akt inhibitors. The *PIK3CA* mutation H1047R, for example, represents an independent predictor for response [30]. This should be taken into account in the selection of patients for studies with PI3K inhibitors; due to the heterogeneous state of the data, the ideal biomarker has not yet been identified.

p110α-Specific Inhibitors

The starting point for p110α-specific inhibitors was the fungal metabolite wortmannin, which leads to powerful and irreversible inhibition of PI3K. The target is the p110 subunit. The inhibition of cell growth was demonstrated in tumor cell lines [31]. The first PI3K inhibitor to be developed was LY294002, which had the same mode of action as wortmannin. In preclinical studies, enhancement of cytotoxic therapies was demonstrated in various tumor entities [32]. PX-866 is a synthetic derivative of wortmannin. Due to an irreversible interaction with the adenosine triphosphate (ATP) binding site, it is a potent PI3K inhibitor. Highly promising results have already been achieved with it in combination with docetaxel in solid tumors [33].

BYL719 (alpelisib) is an oral, class Iα-specific PI3K inhibitor. In vitro, BYL719 has been shown to inhibit the proliferation of cell lines harboring *PIK3CA* mutations. In vivo, BYL719 demonstrated dose-dependent inhibition of tumor growth in various subcutaneous tumor transplant models. BYL719 was subsequently tested in a phase I study in patients with solid tumors who had a *PIK3CA* mutation. A favorable safety profile was reported. Partial remissions were observed in 7 of 39 patients, 2 of whom had ER-positive breast carcinomas. At a dosage of 270 mg/day, 15 patients with ER-positive and HER2-negative breast carcinomas achieved a PFS period of 5.5 months [28].

Combination of alpelisib or a placebo with fulvestrant is currently being investigated in a phase III study in postmenopausal women and in men with HR-positive, HER2-negative advanced breast carcinoma and progression after treatment with an aromatase inhibitor (the SOLAR-1 study) [34]. The effectiveness of BYL719 in comparison with buparlisib or a placebo in combination with letrozole is also being analyzed in a randomized, double-blind study in the neoadjuvant setting. The primary end-point is the assessment of the pathologic complete response (pCR) rate during neoadjuvant treatment in the 2 cohorts (*PIK3CA*-mutated and *PIK3CA* wild-type tumors).

Another agent is currently being evaluated in the framework of the SANDPIPER study [35]. In this phase III study, postmenopausal patients with ER-positive, HER2-negative locally advanced or metastatic breast carcinomas with progression during or after aromatase inhibitor therapy receive treatment with the PI3K inhibitor taselisib (GDC-0032) and fulvestrant, or a placebo and fulvestrant (2:1 randomization).

Pan-PI3K Inhibitors

Initial data on pan-PI3K inhibitors from randomized studies were obtained from the phase III study FERGI, which investigated the addition of pictilisib (GDC-0941; 340 mg/day), a class I PI3K inhibitor, to fulvestrant (500 mg, q28d). A total of 168 patients with ER-positive advanced breast carcinoma who had progressed during aromatase inhibitor therapy were included [36]. The initial results showed a non-significant increase in PFS from 5.1 to 6.6 months (HR 0.74; P = 0.096). In this study, the presence of a *PI3KCA* mutation did not have any predictive value.

Buparlisib (BKM120) is an oral pan-class I PI3K inhibitor that blocks all 4 isoforms of PI3K (α, β, γ, δ) [37]. In a phase Ib study with buparlisib in combination with letrozole in patients with ER-positive, HER2-negative metastatic breast carcinoma (n = 46 with evaluable disease), complete remission and partial remission were observed, as well as stable disease in 25 patients [38]. A correlation with the *PIK3CA* mutation was not identified in the study.

Additional administration of buparlisib in patients with breast carcinoma is currently being analyzed using the BELLE study approaches. The BELLE-2 study is the first randomized phase III study to investigate the safety and efficacy of buparlisib in combination with fulvestrant. A total of 1,147 postmenopausal patients with HR-positive and HER2-negative locally advanced or metastatic breast carcinoma with progression during or after aromatase inhibitor treatment were randomly assigned (1:1) to receive buparlisib (100 mg/day) and fulvestrant (500 mg) or a placebo [39]. The primary end-points of the study are the PFS in the overall group and also in the PI3K-activated group (with *PIK3CA* mutation and/or *PTEN* loss). In all, 53.1% and 52.7% of the patients, respectively, had 1 course of endocrine therapy in the metastatic situation and 19.4% and 22.4%, respectively, had 2 or more courses of endocrine treatment. In the overall group, treatment with buparlisib and fulvestrant was associated with a statistically significant prolongation of PFS by 1.9 months in comparison with placebo and fulvestrant treatment (6.9 vs. 5.0 months; HR 0.79, 95% CI 0.67–0.89; P < 0.01). In the PI3K-activated group, the PFS in the experimental arm was 6.8 months and that in the control arm was 4.0 months (HR 0.76, 95% CI 0.60–0.97; P = 0.014). Data from an exploratory analysis of patients with a *PIK3CA* mutation in circulating tumor DNA (ctDNA; n = 200) are interesting. In those with a confirmed mutation, a PFS of 7.0 months was achieved in comparison with 3.2 months in the placebo arm (HR 0.56, 95% CI 0.39–0.80; P < 0.001). The authors concluded that clinically significant efficacy is achieved, particularly in the subgroup with a *PIK3CA* mutation in ctDNA. In addition, it might be possible to identify a predictive factor for efficacy using a blood sample. The overall survival data are still awaited and will be analyzed after 588 cases of death in the overall group.

The appropriate treatment after combination of an mTOR inhibitor with an aromatase inhibitor represents another clinical challenge. The BELLE-3 study is evaluating the option of treatment with BKM120 in combination with fulvestrant in patients who have progressed after treatment with an aromatase inhibitor and an mTOR inhibitor. Due to the association between resistance to mTOR inhibition and activation of the PI3K/Akt/mTOR signaling pathway, it is assumed that all tumors have an activated PI3K signaling pathway [34].

The BELLE-4 study is a phase II study investigating a combination with weekly administration of paclitaxel.

Dual PI3K/mTOR Inhibitors

The TORC1 inhibitor everolimus is able to eliminate the negative feedback to PI3K activators. On the one hand, this supports the hypothesis that direct inhibition of PI3K is effective while, on the other hand, it has led to the development of dual kinase inhibitors that act on both mTOR and PI3K (e.g., XL765). SAR245409, a PI3K/mTOR inhibitor, is being evaluated in a phase I/II study in combination with letrozole.

PI3K Inhibitors in Combination with Other Therapeutic Agents

As inhibition of PI3K or Akt leads in turn to the up-regulation/activation of numerous tyrosine kinases, combination of PI3K/Akt/TORC1 inhibitors with tyrosine kinase inhibitors may be considered in order to achieve an optimal antitumor effect.

Preclinical and clinical data make it clear that ER-positive tumor cells that are treated with tamoxifen are able to evade the ER blockade via HER2 overexpression [40]. Combined blockade of the ER and HER2 signaling pathways may therefore represent an effective treatment approach.

Synergistic effects between BKM120 and trastuzumab have been demonstrated in HER2-positive cell lines. It was also possible to restore sensitivity to trastuzumab in trastuzumab-resistant xenografts. In a phase I/II study, combined treatment with BKM120 and trastuzumab in patients with trastuzumab-resistant HER2-positive breast carcinomas showed good tolerability, with a clinical benefit of 75%. 17% of the patients had partial remissions and 58% had stable disease after 6 weeks or longer [41].

BKM120 has already been investigated in the neoadjuvant setting in combination with trastuzumab. In the NeoPHOEBE study, patients with HER2-positive primary breast carcinomas of > 2 cm were stratified in accordance with their *PIK3CA* mutation status and randomly assigned after central testing to receive neoadjuvant therapy with 6 weeks of trastuzumab and buparlisib followed by 12 weeks of trastuzumab plus paclitaxel plus buparlisib, or a placebo [42]. The primary end-point was pCR at the time of surgery (ypT0/is). A total of 50 patients were included. Recruitment subsequently had to be stopped due to toxicities and early discontinuations of treatment, as well as an inability to reach the primary study aim. The pCR rates did not differ significantly between the treatments, either overall or relative to the stratified subgroups. The overall response rate (ORR) after week 6 also did not differ between the treatments, although there was a trend toward a better ORR with buparlisib in the ER-positive subgroup (P = 0.053; interaction between buparlisib and ER status, P = 0.032). Buparlisib led to a decrease in Ki-67 from baseline to day 15 in all patients and in the ER-positive subgroup. The authors recommend further investigation of the addition of a PI3K inhibitor to anti-HER2 therapy in the ER-positive and HER2-positive group.

In another study, BKM120 is being investigated in combination with lapatinib in patients with advanced trastuzumab-resistant HER2-positive breast carcinoma and *PIK3CA* or *PTEN* alterations. Preclinical data suggest that cyclin-dependent kinase 4/6 (CDK4/6) inhibition may also have synergistic effects with PI3K inhibitors, in order to reduce the cell survival capacity and overcome intrinsic or secondary endocrine resistance [43]. Phase I/II studies are thus currently investigating a combination of the 2 inhibitors, as in the combination of BYL719 or BKM120 with LEE011 (ribociclib) and fulvestrant or letrozole in postmenopausal women with advanced HR-positive breast carcinomas [16].

Side Effect Profile of PI3K Inhibitors

The PI3K inhibitors are presenting oncologists with a novel side effect profile. According to the phase I studies that have been conducted, the most frequent side effects include gastrointestinal toxicities (diarrhea, nausea, vomiting), skin rashes, hyperglycemia, and also mood changes – which may occur in 1 in 5 patients and may range from anxiety and depression to hallucinations and affective disturbances.

This was also seen in the results most recently presented from the BELLE-3 study mentioned above [39]. Grade III adverse events occurred in 63.2% of cases, and grade IV events, in 14.1% (27.4% and 4.6%, respectively, in the control arm). The most frequent adverse events seen included increased transaminases, hyperglycemia, skin rashes, and also anxiety and depression (22.3% and 26.2%, respectively, for the latter in all grades).

The psychological side effects are probably a result of PI3K inhibition in the central nervous system, as the inhibitors are able to cross the blood-brain barrier. This in turn might offer a potential approach to the treatment of brain metastases. According to the study data, these side effects can be controlled through dose reduction, with or without antidepressant agents or anxiolytics [41].

Conclusions

Endocrine resistance in patients with HR-positive breast carcinoma continues to represent an oncological challenge. Alterations in the PI3K signaling pathway are among the most frequent somatic mutations in ER-positive breast carcinomas, which are associated with endocrine resistance. Numerous PI3K inhibitors are currently undergoing preclinical and clinical development. The data available so far indicate highly promising results.

Disclosure Statement

The authors are investigators in ongoing trials with BYL719 and BKM120.

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