

Development of PI3K inhibitors: lessons learned from early clinical trials

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Abstract | The phosphatidylinositol 3-kinase (PI3K) pathway has an important role in cell metabolism, growth, migration, survival and angiogenesis. Drug development aimed at targetable genetic aberrations in the PI3K/AKT/mTOR pathway has been fomented by observations that alterations in this pathway induce tumour formation and that inappropriate PI3K signalling is a frequent occurrence in human cancer. Many of the agents developed have been evaluated in early stage clinical trials. This Review focuses on early clinical and translational data related to inhibitors of the PI3K/AKT/mTOR pathway, as these data will likely guide the further clinical development of such agents. We review data from those trials, delineating the safety profile of the agents—whether observed sequelae could be mechanism-based or off-target effects—and drug efficacy. We describe predictive biomarkers explored in clinical trials and preclinical mechanisms of resistance. We also discuss key unresolved translational questions related to the clinical development of inhibitors of the PI3K/AKT/mTOR pathway and propose designs for biomarker-driven trials to address those issues.

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Introduction

Activation of the phosphatidylinositol 3-kinase (PI3K) pathway is commonly observed in human cancer and is critical for tumour progression and resistance to anti-neoplastic drugs, including cytotoxic chemotherapy and targeted agents.¹ In addition, PI3K signalling might have a role in non-cancerous cells within the tumour micro-environment.² Several molecular aberrations that affect key components of this pathway, such as the mammalian target of rapamycin (mTOR) or AKT, have prompted the development of PI3K pathway-specific agents.¹ Following the discovery of rapalogues—which inhibit mTOR through an allosteric reaction—and the demonstration of their anti-tumour activity,^{3–6} rationally designed agents that targeted PI3K, AKT and the catalytic domain of mTOR were developed. These second-generation PI3K pathway inhibitors have been evaluated in early clinical trials, and are transitioning to more-advanced clinical development. Partial responses and prolonged disease stabilization have been reported from their use in multiple tumour types, including breast, gynaecological, prostate and lung cancers, and mesothelioma, sarcoma and lymphoma, although at a lower than expected rate.^{7–13} Some of the major challenges in developing agents targeting this pathway have been to determine the pharmacodynamic effects of these inhibitors; to determine whether these agents can inhibit the pathway efficiently, or whether downregulating signalling might be sufficient to produce a clinical response. It is still not known whether downregulation of PI3K alone will demonstrate a similar benefit to other targeted therapies directed against kinases such as KIT,¹⁴ EGFR,¹⁵ or BRAF.¹⁶

Here, we review potential differences among the PI3K/AKT/mTOR pathway inhibitors (PAM inhibitors), and unresolved issues related to these agents that will require further study.

Molecular biology of PI3K in cancer

The PI3K/AKT/mTOR axis regulates essential cellular functions including cell metabolism, growth, migration, survival and angiogenesis (Figure 1). In addition, the role of members of the PI3K/AKT/mTOR pathway—such as PI3K and phosphatase and tensin homolog (PTEN)—in processes such as epithelial-to-mesenchymal transition, DNA repair, autophagy, senescence, and stemness is being investigated.¹⁷

PI3K are divided into three subclasses on the basis of structure, regulation, and lipid substrate specificity.¹ Of these, four different catalytic paralogues (α , β , δ , and γ) compose the class I PI3K and are related to cancer. Class IA isoforms are heterodimeric proteins formed of a p110 catalytic subunit and a p85 regulatory subunit and are involved primarily in the pathogenesis of human cancer. Within class IA, three genes, *PIK3CA*, *PIK3CB*, and *PIK3CD*, encode the homologous p110 α , p110 β , and p110 δ isozymes, respectively. Class IB consists of *PIK3CG*, which encodes p110 γ . Of these proteins, p110 α and p110 β are ubiquitously expressed, whereas p110 δ and p110 γ are mainly found in immune and haematopoietic cells.¹⁷

The oncogenic potential of the PI3K pathway is explained by two key observations. First, alterations in the PI3K/AKT/mTOR pathway can induce cell line transformation and tumour formation in transgenic mice.^{18–20} Second, PI3K signalling activation frequently occurs following multiple molecular alterations in other

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Competing interests

The authors declare no competing interests.

Key points

- Agents targeting different components of the PI3K/AKT/mTOR pathway have been shown to be safe and well tolerated
- These agents inhibit the PI3K/AKT/mTOR pathway at recommended doses, and are effective in multiple tumour types
- No clear correlation among tumour type, genotype and sensitivity to inhibitors of the PI3K/AKT/mTOR pathway has emerged in initial clinical trials of second-generation inhibitors
- Some unsolved questions in the late development of inhibitors of the PI3K/AKT/mTOR pathway might benefit from a systems biology approach and from biomarker-driven studies

components of the pathway downstream of PI3K, such as mutations (*PIK3CA*, *AKT1*, *PTEN*), gene amplifications (*PIK3CA*, *AKT1*, *AKT2*), loss of expression of the tumour suppressors *PTEN* and inositol polyphosphate-4-phosphatase type II, and other mechanisms²¹ that are frequently observed in numerous human tumours (Table 1).¹ In addition, deregulation of the PI3K/AKT/mTOR axis is also observed when upstream oncogenes are mutated or amplified—such as *EGFR*, *HER2*, hepatocyte growth factor receptor (*MET*), fibroblast growth factor receptors (*FGFR1–3*), and *KRAS*—or deletions in other tumour-suppressor genes occur, such as Von Hippel-Lindau disease tumour suppressor (*VHL*), tuberous sclerosis genes *TSC1* and *TSC2*, the threonine-serine protein kinase *LKB1*, neurofibromin (*NF1*) and *TP53*.¹ These mechanisms suggest myriad opportunities for drug development in different types of cancer centred on targetable genetic aberrations in the PI3K/AKT/mTOR pathway.

Clinical development of PAM inhibitors

Drug development of PAM inhibitors has increased during the past decade, as indicated by the number of ongoing clinical trials assessing the numerous compounds targeting PI3K, AKT, and mTOR (Figure 2). Rapalogues were the first inhibitors of PI3K pathway downstream effectors to enter the clinic.²² Although allosteric inhibitors of mTORC1 have shown efficacy as monotherapies, these have only been effective in some neoplasms, such as renal cell carcinoma (everolimus and temsirolimus),^{3,5} neuroendocrine tumours (everolimus),⁶ and mantle-cell lymphoma (temsirolimus).²³ Preclinical studies demonstrated several negative feedback regulatory mechanisms²⁴ and other possible mechanisms of resistance triggered after blockade of mTORC1 by rapalogues, which potentially attenuate the activity of these therapeutic antagonists when used in the single-agent setting. The most impressive data on rapalogues have been generated by combination trials with endocrine therapy in breast cancer. Following the observation that PI3K activation might contribute to resistance to anti-oestrogen treatment,²⁵ anti-oestrogen therapies combined with mTOR inhibitors demonstrated impressive efficacy,^{4,26} engendering the development of second-generation PAM inhibitors.

Current PAM inhibitors in early development (Supplementary Table 1 online) include reversible ATP-competitive inhibitors of the four isoforms of class I PI3K (known as class I PI3K selective inhibitors

or pan-PI3K inhibitors, such as GDC-0941, or XL147); the irreversible pan-PI3K inhibitor PX-866; isoform-specific inhibitors (such as the p110 δ -selective inhibitor CAL-101, and the p110 α -selective INK1117 and BYL719); dual pan-class I–mTOR inhibitors (SF1126, BEZ235, XL765, and GSK1059615); AKT inhibitors (allosteric inhibitors, such as MK-2206, and catalytic inhibitors, such as GDC-0068 and GSK690693); and mTOR inhibitors (rapalogues and catalytic inhibitors such as INK128, AZD8055 and OSI-027).

Dual PI3K–mTOR inhibitors and mTOR inhibitors

The experience with rapalogues led to the development of small molecules designed to inhibit the catalytic site of mTOR. These compounds inhibit mTORC1 and mTORC2 while avoiding the feedback loop mediated by overactivation of AKT.²⁷ The limited antitumour activity of rapalogues is suspected to be related to the fact that these agents only inhibit the mTORC1 complex. Although this limited inhibition could be solved with the use of catalytic inhibitors of mTOR (inhibiting both mTORC1 and mTORC2), expanding the spectrum of antitumour activity, these agents are being developed in the same tumour types in which rapalogues have demonstrated activity (endometrial and kidney cancer, and lymphomas). Other second-generation compounds are dual pan-class I PI3K–mTOR inhibitors. Their dual activity is based on the structural similarities of the catalytic domain of mTOR and the p110 subunit of PI3K, providing the potential advantage of targeting the pathway at two levels (suppressing mTOR in both the mTORC1 and mTORC2 complexes, and PI3K; Figure 1).²⁸

PI3K selective inhibitors

An alternative strategy is the development of ATP-competitive pan-PI3K selective inhibitors. These could be directed at tumours with *PIK3CA* mutations that are ‘addicted’ to the PI3K pathway and whose growth and survival depend on mutations in this specific oncogenic pathway. Although isoform-specific PI3K inhibitors are expected to produce greater target inhibition with fewer adverse effects,^{29,30} the therapeutic window of pan-PI3K and isoform-specific PI3K inhibitors is likely to be limited as they lack selectivity for mutant isoforms. BRAF inhibitors have been developed with selectivity for mutant isoforms,³¹ but this has not yet occurred with PI3K inhibitors.

AKT inhibitors

Some inhibitors of AKT are being tested clinically,^{13,32,33} although the development of AKT-specific and isozyme-selective inhibitors was predicted to be difficult. The anticipated hurdle was the high degree of homology in the ATP binding pocket between AKT, protein kinase A (PKA) and protein kinase C (PKC). GSK690693 and GDC-0068, for example, are ATP-competitive AKT inhibitors that inhibit the three AKT isoforms (AKT1, AKT2, and AKT3). By contrast, allosteric inhibitors seem to be more AKT-specific. For example, MK-2206 is a potent, allosteric non-catalytic pan-AKT inhibitor, and perifosine is an allosteric inhibitor that targets the

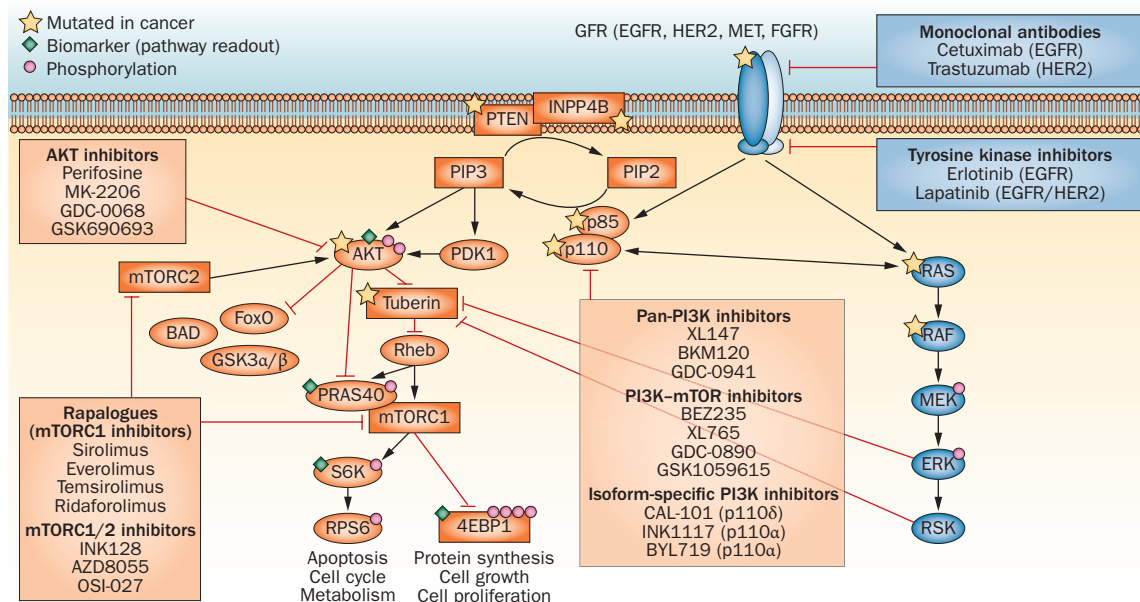


Figure 1 | Signalling of the PI3K/AKT/mTOR pathway and relevant drugs that target each of the components of the pathway. The heterodimer of the PI3Ks (p110 and p85) generates the lipid messenger PIP3, which mediates activation of several protein kinases, including AKT. AKT stimulates glycolysis by activating glycolytic enzymes (via GSK3 β) and by regulating glucose transporters.⁴⁰ This important molecular mechanism drives tumour cells to avidly consume glucose as a source of ATP, also known as the Warburg effect.¹⁰⁸ AKT also promotes survival through BAD and transcription of the antiapoptotic genes, *BIM* and *FASLG* via FoxO. AKT also promotes cell-cycle progression by regulating the cyclin-dependent kinase inhibitors CDKN1A and CDKN1B (also known as p21 and p27) through activation of cyclin D1 and cyclin E1, and the transcription factors JUN and MYC. Protein synthesis, cell growth and proliferation, and metabolic functions downstream of AKT are regulated by the mTORC1/S6K axis and downstream effectors, such as 4EBP1.⁴⁷ Autocrine and paracrine angiogenesis is driven by mTORC1/HIF1 α /VEGF expression. Importantly, negative regulation of this pathway is conferred by PTEN and inositol polyphosphate-4-phosphatase type II, a protein encoded by *INPP4B*, which cleave a phosphate group in PIP3 or PIP2, respectively.¹⁰⁹ Numerous compounds have been developed to inhibit different nodes in the PI3K/AKT/mTOR signalling pathway. These include PI3K inhibitors (that based on their selectivity can be subdivided into dual pan-PI3K-mTOR inhibitors, pan-PI3K inhibitors and isoform-specific inhibitors), mTOR inhibitors (that can be divided into allosteric inhibitors [rapalogues] and mTOR catalytic inhibitors) and AKT inhibitors (including both allosteric inhibitors and catalytic inhibitors). Abbreviations: 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; BAD, BCL2 antagonist of cell death; CDKN1, cyclin-dependent kinase inhibitor 1; *FASLG*, Fas antigen ligand; FoxO, forkhead box O; GFR, growth factor receptor; GSK3, glycogen synthase kinase-3; HIF1, hypoxia-inducible factor 1; *INPP4B*, type II inositol 3,4-bisphosphate 4-phosphatase; mTORC, mTOR complex; PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP2, phosphatidylinositol (4,5)-biphosphate; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PRAS40, proline-rich AKT1 substrate 1; PTEN, phosphatase and tensin homologue; RPS6, 40S ribosomal protein S6; RSK, 90 kDa ribosomal protein S6 kinase.

pleckstrin homology domain of AKT, thereby preventing its translocation to the plasma membrane required for activation.³⁴ The potential role of AKT inhibitors is being investigated. Although some cancers with *AKT1* mutations and *AKT1* and *AKT2* amplifications might be expected to be among the most sensitive to AKT inhibitors, it seems that *PIK3CA* mutant cancer cell lines exhibit only minimal AKT activation and the role of these drugs in this setting should be considered carefully.³⁵

The next wave of drugs targeting the PI3K/AKT/mTOR pathway soon to be tested in the clinic are isoform-specific PI3K inhibitors targeting PI3K β , inhibitors of ribosomal protein S6 kinase beta-1 (S6K), PDK1 inhibitors and isoform-selective AKT kinase inhibitors (thus far, no inhibitors have been developed to target protein kinase AKT1 versus protein kinase AKT2 independently). Figure 1 shows representative examples of early clinical and translational data related to PAM inhibitors and Figure 2 shows the status of their development.

Safety of PAM inhibitors

Overall, the profile of PI3K inhibitors regarding adverse events has been acceptable, with no unexpected toxic effects. Toxic effects have been primarily mild to moderate and manageable with supportive medication.^{7,13} Dose-limiting toxic effects reported with multiple agents include hyperglycaemia, maculopapular rash, gastrointestinal intolerance (anorexia, nausea, vomiting, dyspepsia, diarrhoea), and stomatitis.^{8,36} Although some of these toxic effects are 'off-target' effects, others may be related to target engagement and directly related to mechanisms of action.³⁷ These mechanism-based toxic effects could be used as pharmacodynamic biomarkers and for clinical decision-making, including dose titration in cases in which there are no side effects; for example, dose escalation until the outbreak of rash in the development of EGFR inhibitors.³⁸

Treatment with PAM inhibitors has resulted in hyperglycaemia, which has been manageable with metformin and,

Table 1 | Molecular alterations in the PI3K pathway

Node or alteration	Disease	Frequency (%)
PIK3CA		
Mutation in <i>PIK3CA</i>	Breast cancer	25
	Endometrial cancer	26
	Urinary tract cancer	21
	Colon cancer	12
	Ovarian cancer	10
Amplification of <i>PIK3CA</i>	Head and neck cancer	42
	Squamous cell lung cancer	66
	Gastric cancer	36
	Colon cancer	37.9
	Breast cancer	9
PTEN		
Monoallelic loss of <i>PTEN</i>	Glioblastoma	75
	Colon cancer	20
	Breast cancer	40–50
	Lung cancer	37
	Prostate cancer	42
Biallelic mutation of <i>PTEN</i>	Endometrial cancer	50
	Glioblastoma	30
	Prostate cancer	10
	Breast cancer	5
	Colorectal cancer	7
Loss of expression of <i>PTEN</i> *	Endometrial cancer	NA
	Prostate cancer	NA
	Breast cancer	NA
	Ovarian cancer	NA
	Glioblastoma	NA
Other regulators of <i>PI3K</i>	Melanoma	NA
	Prostate cancer	NA
	Breast (triple negative) cancer	NA
	Ovarian cancer	NA
	Ovarian cancer	NA
Mutation of <i>PIK3R1</i>	Glioma	4
	Colon cancer	3
	Ovarian cancer	<5
AKT		
Mutation of <i>AKT1</i>	Colon cancer	1
	Breast cancer	4
	Ovarian cancer	1
	Endometrial cancer	NA
Amplification of <i>AKT1</i>	Gastric cancer	20
	Breast cancer	1
Mutation of <i>AKT2</i>	Colon cancer	1
Amplification of <i>AKT2</i>	Pancreatic cancer	20
	Ovarian cancer	14.1
	Breast cancer	3
Mutation of <i>AKT3</i>	Melanoma	1.5
Amplification of <i>AKT3</i>	Breast cancer	9.9

*Loss of expression of *PTEN* can be due to mutation, loss of heterozygosity or epigenetic factors, such as promoter hypermethylation or altered expression of microRNAs (miR-21 and others). Abbreviations: INPP4B, type II inositol 3,4-bisphosphate 4-phosphatase; NA, not available; PIK3R1, PI3K regulatory subunit alpha; PTEN, phosphate and tensin homologue.

occasionally, with subcutaneous insulin.³⁹ Hyperglycaemia is a mechanism-based toxicity of PAM inhibitors. Because PI3K is a key component in the insulin signalling pathway, inhibition of insulin signalling leads to insulin resistance

and increased glucose levels in blood.^{39–41} However, a caveat for using hyperglycaemia as a pharmacodynamic biomarker is that there is a compensatory release of insulin (and C-peptide) and other factors, such as diet, that can influence glucose levels.³⁶

Diarrhoea and skin rash have frequently been observed with PAM inhibitors. The skin rash observed in patients treated with PAM inhibitors is erythematous, nonblistering, maculopapular, and not acneiform, differing from the rash observed with EGFR-targeted agents.^{9,13} Interestingly, a similar type of rash—as well as diarrhoea—has been observed in patients treated with other kinase inhibitors, such as sunitinib.⁴² Dual PI3K–mTORC1/2 inhibitors can cause a wider range of off-target adverse effects, such as fatigue and elevated transaminases, but these do not seem to limit their clinical utility.^{8,12,36} Interestingly, some of the expected toxic effects that are frequently associated with rapalogues—such as hyperlipidaemia, pneumonitis and mucositis—are not associated with PAM inhibitors. For example, the prevalence of pneumonitis is approximately 20% in patients treated with rapalogues, but has rarely been described with dual PI3K–mTOR inhibitors.^{8,36} Research on the mechanisms responsible for rapalogue-associated toxic effects, whether they are associated with the immunosuppressant activity of rapalogues, mTORC1 inhibition or their biochemical structure, could help in elucidating the differences among classes of PAM inhibitors.

Biomarkers

Biomarkers of cell signalling

The pharmacodynamic effects of PAM inhibitors on multiple PI3K pathway nodes have been assessed in surrogate tissues, such as peripheral blood mononuclear cells (PBMC), platelet-enriched plasma, skin, and hair, as well as in tumour biopsy specimens (Table 2). PBMC lysates or platelet-rich plasma have been used to investigate the extent of PI3K inhibition with GDC-0068, GDC-0941, GDC-0980, MK-2206 (weekly regimen) and PX-866.^{11,33,43,44} Blood markers are convenient because they facilitate the evaluation of dose–time relationships through serial sampling. Even so, technical and scientific challenges remain that could render these methods insufficiently robust; for example, drug concentrations in blood are expected to be higher than in tissue; therefore, the correlation with tumour effects is not clear. By contrast, human hair has provided a better outcome than skin, and could allow serial sampling for future dose, antitumour response, and duration of effect analyses.^{45,46}

The readouts most frequently evaluated are phosphorylation of AKT at the residues Thr308 (PI3K activity readout) and Ser473 (mTORC2-dependent phospho-epitope); phosphorylation of the AKT substrate AKT1S1 (also known as PRAS40) at Thr246 (AKT activity readout), phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) at Ser65 and Thr70 (mTORC1-activity readout), and phosphorylation of ribosomal protein S6 (RPS6) at Ser240 and Ser244 (mTORC1/S6K-activity readout; Figure 1 and Table 2).

Quantification of a biomarker in proximity to PI3K—such as levels of PIP3—by mass spectroscopy or ELISA (enzyme-linked immunosorbent assay), does not seem feasible in the clinic. AKT assessment would be the next best option but, in our experience, measuring AKT in tissue requires very stringent conditions for sampling and handling to avoid variability in the context of multicentre clinical trials. Thus, assessment of phosphorylation of RPS6, 4EBP1 or pPRAS40 have been preferable in clinical biomarker development.^{7,11,36} Nevertheless, phosphorylation of RPS6 and 4EBP1 can also be regulated by enhanced RAS/RAF/ERK/mTORC1 activity, thereby potentially masking biomarker pharmacodynamic effects.⁴⁷ Other studies have investigated the potential pharmacodynamic effects of PAM inhibitors on Ki-67, a marker of proliferation, and on the apoptotic marker TUNEL.⁹ Biomarker analysis from completed studies reflects a dose-dependent decrease (between 30% and 90%) in markers such as pRPS6, pAKT and 4EBP1 when used at the maximum tolerated dose (MTD) of the different compounds (Table 2), demonstrating target modulation.

Biomarkers of metabolic effect

On the basis of the role of the PI3K pathway in physiological glucose metabolism and of Warburg's observations that tumour cells have a higher rate of glucose consumption, some studies with PAM inhibitors have included biomarkers of metabolic effect.^{41,48} Consequently, assessment of the dose–response relationship has been attempted by measuring fasting glucose, insulin and C-peptide levels in plasma, and glucose uptake using ¹⁸F-fluorodeoxyglucose (FDG)–PET scans.⁷ The assessment of plasma C-peptide has served as a surrogate marker to demonstrate dose-dependent PI3K inhibition, an effect that was observed after only a few days of treatment.^{7,36} Data from early clinical trials demonstrate that measuring C-peptide can be used to delineate a biologically active dose when comparing different dose levels, but diurnal fluctuations confound its use as a biomarker for decision-making for individual patients.³⁶ A decrease in FDG uptake has been observed in several phase I trials following PAM inhibitor treatment.^{7,11,49} Whether the cause of this decrease is related to a pharmacodynamic effect of PI3K inhibition (the role of PI3K signalling in glucose uptake) or an anti-tumour effect is not known yet, and in some cases, both can have a role, as seen with mTOR inhibitors.^{50,51}

Signs of activity and predictive biomarkers

Signs of antitumour activity (partial responses and prolonged disease stabilization with minor tumour shrinkage or tumour marker reduction) have been reported in the phase I trials of the first PAM inhibitors, including breast, non-small-cell lung, gynaecological, pancreatic, bladder, anal, and prostate cancer, renal cell carcinoma, melanoma, mesothelioma, sarcomas and lymphomas.⁵² With few exceptions, the clinical efficacy of many PI3K pathway inhibitors as a monotherapy is modest at best. However, the selective agent, CAL-101—a highly specific PI3K δ inhibitor—has shown significant clinical activity in several lymphoid malignancies.¹⁰

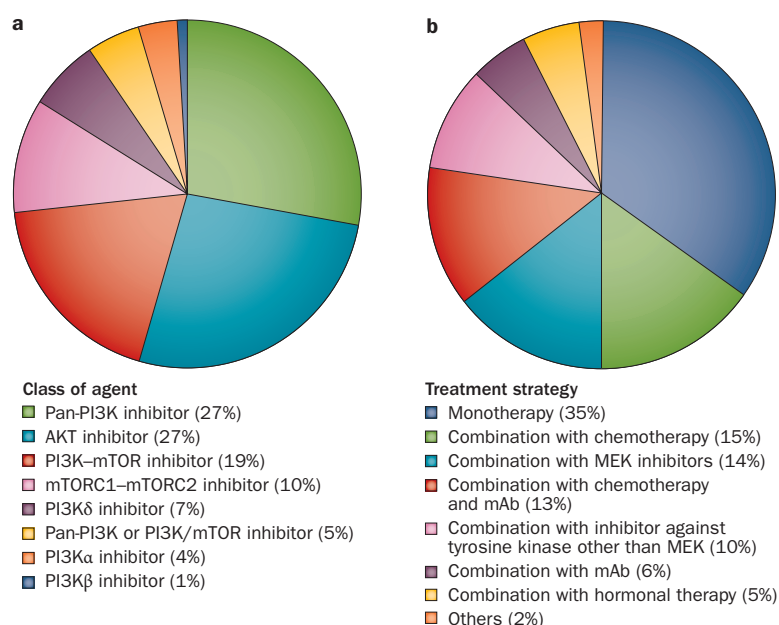


Figure 2 | Distribution of ongoing clinical trials depending on the class of agent or treatment strategy. More than 50 new drugs inhibiting the PI3K/AKT/mTOR pathway are at different stages of development (Supplementary Table 1 online). The data depicted in this figure were obtained from ClinicalTrials.gov in November 2012. **a** | Most drugs in development are pan-PI3K, PI3K–mTORC1/2 or AKT inhibitors and only 12% comprise isoform-specific PI3K targeting agents. **b** | PI3K/AKT/mTOR inhibitors are being assessed as single agents mainly in histology-driven trials, such as breast cancer, non-small-cell lung cancer, gastrointestinal and gynaecological malignancies, prostate cancer and glioblastomas. Approximately two-thirds are testing combination approaches, and of these, around 30% of ongoing phase Ib–II trials with PI3K/AKT/mTOR inhibitors are investigating combination regimens with chemotherapeutic agents. Abbreviation: mAb, monoclonal antibody.

One strategy to increase monotherapy response rates is to improve the selection of patients with known aberrations and mutations. Preclinical findings showing that tumours with PI3K pathway alterations are more sensitive to treatment with PI3K inhibitors than tumours without such alterations have encouraged the enrichment of trials with patients whose tumours harbour mutations in *PIK3CA* and *PTEN*, or have lost expression of *PTEN*.^{53–56} However, initial clinical trials with PAM inhibitors have shown no clear correlation between molecular alterations in the PI3K pathway and antitumour effect;^{7,13,44} although some pooled analyses have suggested that there is a correlation,⁵⁷ others have suggested otherwise.⁵⁸ Moreover, partial responses and significant tumour shrinkage were observed in *KRAS* mutant breast, ovarian and pancreatic cancer as well as *BRAF*-mutant melanoma patients following treatment with a PAM inhibitor,^{11,13,36,59} an unexpected finding as tumours with activating mutations in members of the MAPK pathway may potentially be resistant to PAM inhibitors.^{60–63}

Once a recommended dose was established in some phase I studies (such as those studies assessing the inhibitors BKM120 and BEZ235), preselected patients with solid tumours and with PI3K pathway alterations were enrolled in the expansion phase of those studies

Table 2 | Pharmacodynamic markers explored in the first clinical trials with PI3K/AKT/mTOR inhibitors

Agent	PD imaging FDG-PET	PD effects on surrogate tissue (% of decrease from baseline)	PD effects on tumour tissue (% of decrease from baseline)
Pan-PI3K			
XL147 ⁹	Not presented	Decreased levels of pRPS6 in skin (in selected cases)	Decreased levels of pAKT Thr308 (40–80%), p4EBP1 (60–70%), pERK (40–60%), and Ki-67 (in selected cases)
BKM120 ⁷	Yes (9/19 patients had a metabolic PR)	Decreased levels of pRPS6 in skin in >40% patients (11/14 at MTD); increased levels of C-peptide (dose-dependent)	Decreased levels of pRPS6, pAKT, p4EBP1 and Ki-67 (in selected cases)
GDC-0941 ¹¹	Yes (6/17 patients had a metabolic PR)	Decreased levels of pAKT Ser473 in PRP	Decreased levels of pRPS6 (in selected cases)
BAY80-6946 ⁴⁹	Yes (selected cases)	Not presented	Not presented
PX-866 ⁴⁴	Not presented	Decreased levels of RPS6 ribosomal protein and mTOR phosphorylation in PBMCs	Not presented
CH5132799 ¹¹⁰	Yes (selected cases)	Decreased levels of pAKT in PRP (up to 80%)	Not presented
PI3K-mTOR			
XL765 ⁸	Not presented	Not presented	Decreased levels of pAKT Thr308 (50–80%), p4EBP1 (60–80%), and pERK (50–80%) in selected cases
BEZ235 ³⁶	Yes (8/37 patients had a metabolic PR with QD dosing and 4/9 with BID dosing)	Increased levels of C-peptide, and decreased levels of pRPS6 in skin and sVEGFR2 (dose-dependent)	Decreased levels of pRPS6 (in selected cases)
GDC-0980 ¹²	Yes (5/6 patients had a metabolic PR)	Decreased levels of pAKT (>90% compared with baseline)	Not presented
mTORC1/2			
OSI-027 ¹¹¹	Not presented	Decreased levels of p4EBP1 Thr37/Thr46 in PBMCs (>60% compared with baseline)	Not presented
AZD2014 ¹¹²	Not presented	Decreased levels of pAKT Ser473 in PRP, p4EBP1 Thr37/Thr46 in PBMCs	Decreased levels of pRPS6 Ser235/Ser236 (average 38%, 8/10 samples), pAKT Ser473 (average 40%, 3/6 samples), and Ki-67 (selected cases)
CC-223 ¹¹³	Not presented	pRPS6 (B cells), p4EBP1(T cells) and pAKT (monocytes)	Not presented
MLN-128 (INK128) ¹¹⁴	Not presented	Decreased levels of p4EBP1 in PMBCs, and decreased levels of p4EBP1, pRPS6 and pPRAS40 in skin in 60–100% of patients	Not presented
AKT			
MK-2206 ¹³	Not presented	Sustained decreased levels of pPRAS40 Thr246/total PRAS40 ratio (median 48%) in hair follicles (QD regimen)	Decreased levels of pAKT Ser473 (40–95%, median 89%, in 9 patients at MTD-QD regimen)
GDC-0068 ³³	Not presented	Decreased levels of pGSK3β in PRP (dose-dependent) of >70% compared with baseline	Decreased levels of pPRAS40 (>50%) and cyclin D1 (dose-dependent)
GSK795 ³²	Yes (7/8 patients had a metabolic PR)	Not presented	Decreased levels of pPRAS40 (30–70%), Increased levels of pAKT (in selected patients)
PI3Kδ			
CAL-101 ¹⁰	Not presented	Decreased levels of pAKT Thr308 in CCL cells of >90% compared with baseline	Not presented

Abbreviations: BID, twice a day; CCL, chronic lymphocytic leukaemia; FDG-PET, ¹⁸F-fluorodeoxyglucose PET; MTD, maximum tolerated dose; p4EBP1, phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1; PBMCs, peripheral blood mononuclear cells; PD, pharmacodynamic; pGSK3β, phosphorylation of glycogen synthase kinase-3 beta; PR, partial response; PRP, platelet-rich plasma; pPRAS40, phosphorylation of proline-rich AKT1 substrate 1; pRPS6, phosphorylation of 40S ribosomal protein S6; QD, every day; QW, once a week; sVEGFR2, soluble VEGFR2.

to test the value of these alterations as predictive biomarkers.^{7,64} Although no data are yet available to show whether clinical responses are higher in this molecularly selected patient population, the response rates in selected versus unselected patients have not, in our experience, been markedly different. Despite the fact that selectivity towards a population harbouring mutations in PI3K has not been proven, several phase I studies assessing isoform-specific PI3K (such as BYL719 and INK1117) have restricted enrolment to only patients

with *PIK3CA*-mutant tumours. Preliminary results of the first-in-human study of BYL719 have shown some objective responses and prolonged disease stabilization with tumour shrinkage in breast (among *PIK3CA*-mutant patients with oestrogen-positive breast cancer, 33% achieved a tumour shrinkage of more than 20%), colorectal (one partial response) and gynaecological malignancies (one partial response).⁵⁹

The inconclusive predictive value of *PIK3CA*, *PTEN*, *KRAS* or *BRAF* mutations for delineating the clinical

value of PAM inhibitors may be due to several reasons. First, tumours without PI3K alterations might have responded because early detection methods were based on a limited number of assays and were unable to detect other alterations that could be driving sensitivity such as alterations in *AKT1/2*, *PIK3R1*, *LKB1*, or *NF1*, or were using improper assays or thresholds (for example, detection of PTEN loss lacks a well-defined threshold for immunohistochemistry categorization and neither the antibodies used for immunohistochemistry nor the criteria for assessment are uniform among different laboratories). Second, tumours described as having PI3K alterations might have not responded because of the coexistence of mutations related to resistance, such as mutation in *KRAS*,^{61,62} intratumour heterogeneity,³⁵ or the use of incorrectly standardized or unvalidated assays. Finally, there are conflicting preclinical data^{55,65,66} regarding the preferential activity of PAM inhibitors in specific molecular scenarios.

In summary, it seems that PAM inhibitors in monotherapy have not shown the same level of success as targeted therapies, such as kinase inhibitors directed towards ALK and BRAF. Clinical responses to PAM inhibitors as monotherapy are less frequent than had been expected and it seems that translating PAM alterations to the clinic as predictive markers for patient selection might not be as straightforward as originally hoped.

Stop and think: unresolved questions

After the first round of clinical trials with second-generation PI3K pathway inhibitors, several sets of important questions remain unanswered and may determine the future development of these agents (Box 1).

Pharmacological questions

Because most phase I trials with these agents are considered 'completed' and these agents are now in the later stages of development, the pivotal unanswered questions are related to unsolved pharmacological issues. First, on the basis of clinical activity and toxicity profiles known to date, what remains to be defined is whether current PI3K, AKT or mTOR1/2 inhibitors are optimal drugs, and if there is a specific genetic context that prognosticates superior activity for each class. The magnitude of signalling inhibition required to produce biological and clinical effects has not been clearly delineated either. Whereas responses to selective BRAF inhibitors in melanoma were well correlated with an inhibition of intratumoural pERK pathway activity >80%, the same level of certainty is not available in the case of PI3K inhibitors.⁶⁷ Based on the observed pathway inhibition (in the 30–90% range depending on the marker and the drug assessed), it is not known if these drugs when administered at the MTD are inhibiting the pathway sufficiently to have an antitumour effect. Off-target toxic effects—or an incorrect assessment and management of on-target, mechanism-based toxic effects—could preclude attaining profound pathway inhibition. Therefore, questions remain as to whether a therapeutic window can be achieved without inducing deleterious side effects, such

Box 1 | Unanswered questions in the development of PI3K/AKT/mTOR inhibitors

Pharmacological issues

- What extent and duration of PI3K inhibition in tumours is necessary for antitumour activity? Is it better to inhibit the pathway continuously or with a more-potent intermittent approach?
- Are there clinically significant differences among different PI3K pathway inhibitors?
- What are the tumour biology and tumour environment consequences of PI3K inhibition (for example, apoptosis, reduction in cell proliferation or angiogenesis inhibition) with each class of agent? Do they depend on a specific genetic context? Which are the best pairs of drug class–tumour genotype for PI3K-pathway inhibition?
- How do we select biologically active doses? What is the best readout of pathway inhibition? Are surrogate tissues good proxies for evaluating drug effects in tumour or only drug exposure?

Patient-selection issues

- Are mutations in different nodes of the pathway (that is, *PTEN* mutation versus *PIK3CA* mutation versus *AKT* mutation) equivalent in terms of predictability of sensitivity?
- What is the impact of PI3K inhibition on tumour cell growth in patients with no activating mutations compared to those with molecular evidence for PI3K pathway activation (for example, *PIK3CA* mutations, *PTEN* loss, *HER2* overexpression)?

Systems biology issues

- Do different PI3K isoforms have diverse roles in different tissue types, context or tumours?
- Does the same mutation have the same response in different cancers or does its role depend on cancer type or context? Are there relevant differences in pathway alterations between primary and metastatic sites?
- What are the mechanisms of resistance to PI3K inhibitors? Are all described feedback loops present in all tumour types or are they context-dependent? Are any of those described in preclinical models clinically significant and epidemiologically relevant?
- Is the use of PI3K/AKT/mTOR inhibitors in monotherapy rational? Or should we use them only in combination with other agents, for overcoming cross-talk and resistance or to enhance the efficacy of cytotoxic, hormonal or targeted agents?

as insulin resistance, and if this toxicity is acceptable in the context of the antitumour activity produced by PI3K inhibitors. Interestingly, some preclinical studies have shown that modulation of AKT is necessary, but not sufficient, to ensure tumour growth inhibition.⁶⁸ These uncertainties pose unanswered questions regarding the therapeutic index of PAM inhibitors and their recommended phase II doses: should we select the MTD or the biologically active dose? Probably most important: would it be best to inhibit the pathway continuously or with a more-potent intermittent approach?

An opportunity for combinations

Because the PI3K pathway comprises a complex network of interactions with parallel cascades, its pharmacological inhibition releases negative feedback resulting in activation of compensatory signalling pathways. Among these compensatory mechanisms are a FOXO-dependent feedback reactivation of receptor tyrosine kinases (such as *HER2*,⁶⁹ *HER3*,⁷⁰ *IGF1R*, and insulin receptor⁷¹) and downstream kinases including *ERK*,^{69,72} *MYC* amplification, and *NOTCH*^{73–75} or Wnt– β -catenin pathway activation.⁷⁶ Although these findings derive from *in vitro* experiments and indicate resistance to PI3K inhibition, further clinical validation is needed for more-definitive conclusions.

To overcome negative effects on PI3K pathway inhibition, *in vivo* combination therapy with receptor tyrosine kinase inhibitors (such as those directed against EGFR, HER2, or HER3) resulted in decreased proliferation, enhanced cell death and superior antitumour activity compared with single-agent PI3K inhibitors.^{69–71} Some of these combination strategies, such as trastuzumab or lapatinib in combination with PAM inhibitors, are being tested in ongoing clinical trials.^{77,78}

Using horizontal combination approaches that include PAM inhibitors and MEK inhibitors has been proposed to overcome the parallel induction of the MAPK pathway by PAM inhibitors.⁷⁹ These combinations of small-molecule kinase inhibitors might, however, be challenging, particularly because of the increased levels of toxicity associated with them (the rates of drug-related grade 3 or 4 adverse events are around 18.1% for monotherapy and approximately 53.9% when combining a MAPK and a PI3K inhibitor, with transaminase elevations, skin rash, and mucositis the most frequent ones),⁸⁰ which could require using inadequate doses that do not provide sufficient dual pathway inhibition.^{81–84}

Cell signalling changes resulting from PI3K alterations might not be limited to activation of receptor tyrosine kinases and MAPK pathway. In hormone-sensitive tumours, such as prostate and breast cancer, PI3K pathway alterations are triggered as resistance mechanisms to hormone therapies. In PTEN-deficient prostate cancer cells, PI3K and androgen receptor (AR) cross-regulate each other by a reciprocal feedback mechanism (inhibition of the AR activates AKT signalling, whereas inhibition of PI3K results in feedback signalling to HER2–HER3 and activation of AR), which is abrogated by combined pharmacological inhibition.¹⁸ Clinical trials combining androgen deprivation therapy with PAM inhibitors are currently recruiting patients.^{85–87} A similar phenomenon is observed in oestrogen receptor-positive breast cancer cells.⁸⁸ Following the positive results of the BOLERO-2 trial of everolimus administered in combination with exemestane, many PAM inhibitors in combination with anti-oestrogen therapies are undergoing clinical evaluation in endocrine-sensitive breast cancer.⁸⁹ Preclinical studies have also suggested that PI3K modulation could overcome resistance to chemotherapy agents, such as platinum-based drugs and taxanes, doxorubicin and 5-fluorouracil.⁹⁰ In preclinical models, concomitant inhibition of the PI3K pathway with chemotherapy was shown to enhance the efficacy of some agents by decreasing the phosphorylation of AKT that can be raised in response to insult as a survival mechanism.^{91,92} However, this synergistic effect is not universally true. Inhibition of the PI3K pathway results in arrest in the G1 phase of cell cycle, which could decrease antitumour effect of agents whose activity induces arrest in later cell cycle phases.^{93–95} Approximately 30% of ongoing phase Ib–II trials using PAM inhibitors are investigating combination regimens that include chemotherapeutic agents (Figure 2). Preliminary results of ongoing trials with GDC-0941, PX-866 and GDC-0068 added to taxanes and platinum agents showed good overall tolerance

at the MTD of both agents and responses were seen in multiple tumour types.^{96–98}

Translational efforts to move forward

Given the moderate clinical benefit observed after exposure to PAM inhibitors as monotherapy, patients with demonstrated clinical responses and their tumour biopsies have become tremendously valuable as a vehicle to identify mechanisms of sensitivity. Additionally, on-treatment tumour biopsies would be useful for analysing mechanisms of action and defining an optimal biological dose. Therefore, we believe that including on-treatment biopsies in upcoming clinical trials is crucial for the further development of PAM inhibitors.

Other questions—such as what should be used as a predictive marker or how to match a drug target with a predictive marker—are also important and complex enough to warrant testing in specially designed proof-of-concept trials.⁹⁹ Tissue-based novel phase II designs can provide information that can be used to design phase III trials. Biomarkers in different genetic contexts (such as the effect of the drug on kinase inactivation, gene expression, cell proliferation, apoptosis, and inhibition of the drug target *in situ* [by assessing pAKT, pPRAS40, pRPS6, and others]) can be studied in biomarker-driven trials (with paired biopsies) or in the neoadjuvant setting.¹⁰⁰ If found, these answers could be used later for patient selection or exclusion from registration trials. This approach was feasible and useful in exploring the effect of the aromatase inhibitor letrozole combined with everolimus.^{4,101}

Identifying reliable biomarkers for patient selection is of paramount importance. Tumour-based biomarkers could be expanded not only to target genomic aberrations (*PIK3CA*, *PTEN*, *AKT1/2*, *PIK3R1*, *LKB1* and *NF1*), but also to include ‘PI3Kness’ status, which might serve as a readout of all activation alterations.¹⁰² In addition, non-tissue-based biomarkers are rapidly developing. One approach would be to determine noninvasively current mutation status in circulating DNA found in the plasma of patients with metastatic disease. This assessment is particularly important because the mutational pattern of tumours can change at the time of disease recurrence.¹⁰³

Lacking a qualified and defined drug target–predictive marker relationship,¹⁰⁰ one could opt for an ‘all-comers’ approach with a prespecified retrospective assessment of potential biomarkers. Advantages of a preselected patient population (enrichment approach) are, however, straightforward resulting in proof-of-concept, quick determination of responsive patient population and anticipated clinical benefit if the preclinical hypothesis holds true.¹⁰⁴

In disease settings for which the benefit of first-generation mTOR inhibitors has been defined, direct comparison with second-generation agents (or combinations, based on solid preclinical data) is also appealing. Therefore, research efforts will benefit from addressing the differential effects, if any, of each class and the genetic contexts to which different drugs are best suited. An interesting approach for clinically testing PAM inhibitors in breast cancer, for example, is the ‘add-on phase II trial design’, in which patients on standard therapy (such

as anti-HER2 or endocrine therapy) undergo biopsies at the time of disease progression and then receive different matched targeted agents that are expected to reverse resistance with each patient acting as her own control.

With the plethora of available agents, it seems daunting to address all related questions through clinical trial testing. This field will benefit greatly from a systems biology approach, the development of patient-derived xenografts that allow co-clinical trials, and current developments in bioinformatics and public databases.^{105–107}

Conclusions

On the basis of the considerations described in this Review, it is reasonable to state that PAM inhibitors have the potential to inhibit the PI3K/AKT/mTOR pathway with reasonable efficacy and a favourable safety profile. The observed antitumour activity of mTOR inhibitors as monotherapy or in combination, and the potential indications that could be explored based on the frequency of alterations in the pathway clearly encourage further investigation with PAM inhibitors. To succeed in the late development of these agents, pharmacological, biological and translational issues need to be examined to better understand how to appropriately apply these agents.

Remarkably, each agent class and compound seems to be slightly different regarding its biological effect, but it is unknown whether mTOR kinase, AKT, pan-PI3K, or isoform-specific PI3K inhibitors will provide the greatest therapeutic index, or whether they will need to be

combined with inhibitors of receptor tyrosine kinases or downstream targets. It is likely that the applicability of the different agent classes may depend on tumour type, genotype and therapeutic index of the combinations. Innovative, biomarker-driven studies are needed to answer some of these questions, avoiding as much as possible the serendipitous and empirical approaches that have been used in the development of many targeted therapies in the past.

Review criteria

The PubMed database was searched for articles published in English before October 2012. The search terms included: “PI3K”, “mTOR”, “AKT”, “cancer”, “clinical trial”. We considered articles published only in English and selected all the trials that included a PI3K/AKT/mTOR (PAM) pathway inhibitor. We also selected preclinical papers with the most robust and referenced data. Finally, we also considered abstracts released at the AACR and ASCO annual meetings and EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics in 2010, 2011, and 2012. The ClinicalTrials.gov website was searched for ongoing clinical trials with PAM inhibitors. This Review includes a summary of the authors’ work and knowledge based on participating in more than 10 phase I trials with PAM inhibitors. Knowledge gained from reading the oncology literature and regular attendance at conferences, workshops, and other national and international meetings was also included.

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Author contributions

All the authors researched the data for the manuscript, made a substantial contribution to discussion of content, wrote and reviewed and edited the manuscript before submission.

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