Targeting PI3K signalling in cancer: opportunities, challenges and limitations

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Abstract | There are ample genetic and laboratory studies that suggest the PI3K–Akt pathway is vital to the growth and survival of cancer cells. Inhibitors targeting this pathway are entering the clinic at a rapid pace. In this Review, the therapeutic potential of drugs targeting PI3K–Akt signalling for the treatment of cancer is discussed. I focus on the advantages and drawbacks of different treatment strategies for targeting this pathway, the cancers that might respond best to these therapies and the challenges and limitations that confront their clinical development.

Despite improvements in cancer therapies over the past 50 years, metastatic solid cancers remain largely incurable, and the survival for patients with these malignancies is often measured in months. In this era of targeted therapies, substantial efforts are being made to identify the optimal target for each type of cancer. These have been spurred by the few successes, such as imatinib for chronic myelogenous leukaemia (CML); trastuzumab for breast cancer with amplification of *ERBB2* (also known as *HER2*); and erlotinib and gefitinib for lung cancer that expresses mutant epithelial growth factor receptor (*EGFR*). Accumulating genetic and cancer biology studies indicate a prominent role for the PI3K pathway in cancer cell growth and survival, and have culminated in the aggressive development of PI3K pathway inhibitors as cancer therapies. In this Review, I will evaluate the different strategies for inhibiting this pathway. In addition, I will examine which cancers will most likely respond to PI3K pathway inhibitors, the design of promising combination therapies and strategies to improve the clinical development of these compounds. As PI3K pathway inhibitors are currently in early-phase clinical trials, these considerations seem particularly relevant at this crucial junction in their evolution.

There have been several reviews on the molecular mechanics of PI3K signalling and the resulting signalling networks that promote cell growth and survival[1–7]. Therefore, these signalling networks will be reviewed only briefly here. The PI3K family of lipid kinases phosphorylate the 3′OH group of phosphatidylinositols. There are three classes of PI3K, each with its own substrate specificity and distinct lipid products (reviewed in REFS 1,3). The Class I_A of PI3Ks is the most widely implicated class in cancer and will be the focus of this Review. It is described in more detail in BOX 1. PI3K activation initiates a signal transduction cascade that promotes cancer cell growth, survival and metabolism. Akt, a serine–threonine kinase that is directly activated in response to PI3K, is a major effector of PI3K in cancers. There are three different Akt isoforms in mammalian cancers, and emerging data suggest that they have overlapping and distinct roles in cancers. As shown in BOX 1, Akt signalling leads to increased cellular growth and survival. Although Akt is the PI3K effector that is most widely implicated in cancer, there are Akt-independent pathways activated by PI3K, which include the Bruton tyrosine kinase (BTK); the Tec families of non-receptor tyrosine kinases; serum- and glucocorticoid-regulated kinases (SGKs)[8]; and regulators of small GTPases that are implicated in cell polarity and migration[9]. However, the roles of these Akt-independent pathways in human cancer are currently less well defined and they will not be discussed in detail.

One of the major effectors downstream of Akt is mTOR complex 1 (mTORC1). As described in BOX 2, mTORC1 is often not only under the control of PI3K–Akt signalling. mTORC1 integrates many inputs, including growth factor signalling, the energy state of the cell (that is, AMP levels) and nutrient and O_2 availability (BOX 2). From a therapeutic perspective, the complex regulation of mTORC1 is important, as some PI3K inhibitors in development directly block both PI3K and mTOR, whereas others inhibit only PI3K (TABLE 1). As will be discussed in more detail below, dual PI3K–mTOR inhibitors might offer a therapeutic advantage in cancers in which PI3K is not the main regulator of mTORC1.

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doi:10.1038/nrc2664

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At a glance

- There are several therapeutics that target the PI3K–Akt pathway in clinical development for the treatment of cancer. These include dual PI3K–mTOR inhibitors, PI3K inhibitors, Akt inhibitors and mTOR complex catalytic site inhibitors.
- The PI3K–Akt pathway is inappropriately activated in many cancers. The pathway is activated by receptor tyrosine kinases, as well as by the genetic mutation and amplification of key pathway components.
- The most effective type of therapeutic used to inhibit this pathway is likely to depend on the particular mechanism of PI3K–Akt activation in a cancer.
- So far, preclinical data suggest that PI3K–Akt pathway inhibitors might have single-agent activity in breast cancers with *ERBB2* amplifications or *PIK3CA* mutations. These drugs might also be effective in overcoming acquired resistance to therapies that target receptor tyrosine kinases (such as acquired resistance to trastuzumab or erlotinib).
- Drugs targeting the PI3K–Akt pathway might most effectively treat cancers when they are used in combination with other targeted therapies, such as MEK inhibitors.
- Effective clinical development will centre on determining why these compounds fail when they do. It will be important to determine whether a drug could not effectively downregulate PI3K–Akt signalling or if effective inhibition of the pathway was not sufficient to produce a clinical response.

Mechanisms of PI3K–Akt activation in cancer

The PI3K–Akt signalling pathway is inappropriately activated in many cancers. To date, the two most widely observed mechanisms of PI3K–Akt activation in human cancers are activation by receptor tyrosine kinases (RTKs) and somatic mutations in specific components of the signalling pathway. Importantly, the mechanism of PI3K pathway activation will affect both the most effective therapeutic approach and the likelihood of clinical benefit from PI3K inhibition.

RTK signalling. The activation of class I_A PI3Ks is clearly linked to RTK signalling. Analogous to the original discovery that the polyomavirus middle T antigen requires a physical interaction with PI3K for its transforming activity[10], RTK-mediated activation of PI3K seems to be crucial for its oncogenic activity. The p85 regulatory subunit is vital in mediating class I_A PI3K activation by RTKs. The Src homology 2 (SH2) domains of p85 bind to phosphotyrosine residues in the sequence context pYxxM (in which 'pY' indicates a phosphorylated tyrosine on activated RTKs or on adaptor molecules[11,12]) (BOX 1). Cancers that exhibit oncogene addiction to an RTK have PI3K activity that is strictly controlled by that RTK. Indeed, for an RTK inhibitor to be an effective therapy, it must lead to downregulation of PI3K signalling[12–17]. In some cancers, multiple RTKs activate PI3K and these cancers are invariably resistant to a single RTK inhibitor[18–20]. In addition to binding phosphotyrosine proteins, PI3K binds directly to Ras[21,22]. However, it remains undetermined if mutated Ras is sufficient to directly activate PI3K and thereby bypass its usual obligate engagement with phosphotyrosines. Although holoenzymes that contain p110β are also activated by G protein-coupled receptors[23,24], the prominence of this activation mechanism in cancer remains less well defined.

Oncogene addiction
A cellular condition in which a cancer cell requires the activity of a specific oncogene or cellular process for growth and survival. Inhibition of that specific function leads to cell death.

Genetic activation. Several genetic abnormalities are known to activate PI3K–Akt signalling (TABLE 1). The first genetic mechanism identified was loss of the *PTEN* tumour suppressor, which encodes a phosphatidylinositol-3,4,5-trisphosphate (PIP_3) 3′-phosphatase that turns off the PI3K pathway[25–28] (reviewed in REF. 29) (BOX 1). Heterozygous loss of *Pten* in mice results in neoplasia of multiple epithelia, including the intestine, prostate, endometrium and mammary gland[30]. Homozygous deletion of *Pten* in the prostate epithelium leads to aggressive prostate carcinoma[31,32]. Indeed, studies suggest that loss of PTEN expression is associated with higher Gleason scores in primary disease, and that there is an accumulation of *PTEN* mutations in metastases[29]. Although loss of PTEN is tumorigenic, it is unclear if PTEN loss alone is sufficient to activate PI3K. Indeed, recent studies have shown that RTK inhibitors can downregulate Akt even when PTEN expression is lost[18]. Although loss of PTEN might not absolutely preclude the capacity for RTK inhibitors to shut off PI3K signalling, it seems to reduce the likelihood of cancers responding to these therapies as single agents[13,16,17,33].

More recently, somatic activating mutations were identified in the class I_A PI3K catalytic subunit, p110α (encoded by *PIK3CA*)[34]. There have been several reviews discussing these mutations and so they will be discussed only briefly here[2]. Somatic mutations in *PIK3CA* occur in up to 30% of some types of common epithelial cancer, which includes breast, colon, prostate and endometrial cancers (see Further information for a link to the website for the Catalogue of Somatic Mutations in Cancer). It is not yet clear whether *PIK3CA* mutations are early or late genetic events in cancer progression. *PIK3CA* mutations occur more frequently in colorectal cancers than in precursor polyps[34,35]. However, a recent study of *in situ* and invasive breast cancers suggests that *PIK3CA* mutations arise before the development of an invasive phenotype[36]. Most mutations (~80%) reside in one of two hotspot regions in the kinase domain and the helical domain. These mutant p110α subunits increase *in vitro* lipid kinase activity, maintain PI3K–Akt signalling under conditions of growth factor deprivation and can transform cells. Recently, we found that the expression of the kinase domain mutant H1047R of p110α in mouse lungs induced adenocarcinomas *in vivo*[37].

The two classes of *PIK3CA* mutations promote constitutive PI3K signalling through distinct mechanisms. In the wild-type PI3K holoenzyme, p85 inhibits p110α through an intermolecular interaction, and this inhibition is relieved by a conformational change that is induced by the engagement of the p85 amino-terminal SH2 domain with phosphotyrosines[38]. X-ray crystal data and molecular modelling studies suggest that the helical domain mutants E545K and E542K abrogate this inhibitory intermolecular interaction between p85 and p110 (REFS 39, 40). Accordingly, the activity of the helical domain p110α mutant was not increased by the presence of tyrosine phosphorylated peptides *in vitro*[41]. The kinase domain mutant H1047R is located near the activation loop and seems to promote constitutive PI3K signalling through a different mechanism.

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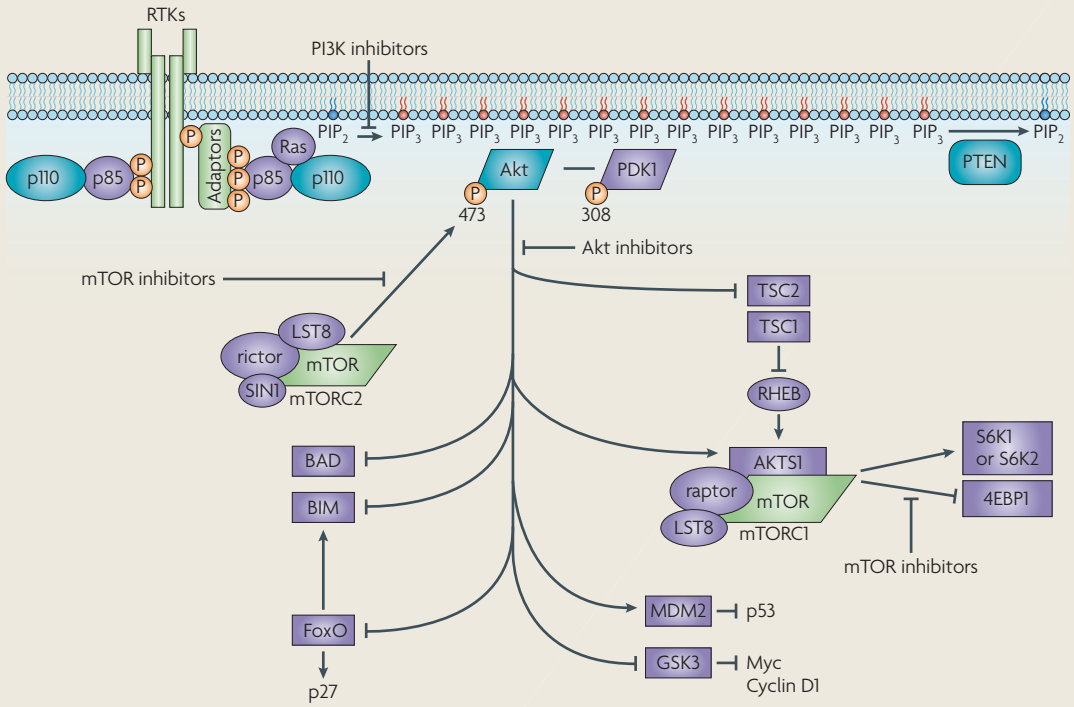
Box 1 | **Class I_A PI3K signalling**



Class I_A PI3Ks primarily phosphorylate phosphatidylinositol-4,5-bisphosphate (PIP_2) on the plasma membrane to generate the second messenger, phosphatidylinositol-3,4,5-trisphosphate (PIP_3). Class I_A PI3Ks are heterodimers that consist of a p85 regulatory and a p110 catalytic subunit. There are several isoforms of both the catalytic (p110α, p110β and p110δ) and regulatory (p50α, p55α, p85α, p85β and p55γ) subunits. Class I_A PI3Ks are most often activated by receptor tyrosine kinase (RTK) signalling, although the p110β-containing enzymes might also be activated by G protein-coupled receptors[23,24]. The p85 regulatory subunit is crucial in mediating class I_A PI3K activation by RTKs. The Src-homology 2 (SH2) domains of p85 bind to phosphotyrosine residues in the sequence context pYxxM (in which a 'pY' indicates a phosphorylated tyrosine) on activated RTKs, as in the case of platelet-derived growth factor receptors, or on adaptor molecules, such as ERBB3 or GRB2-associated binding protein 1 (REF. 11). This binding of SH2 domains serves both to recruit the p85–p110 heterodimer to the plasma membrane, where its substrate PIP_2 resides, and to relieve basal inhibition of p110 by p85 (REF. 38). The 3′-phosphatase PTEN dephosphorylates PIP_3 and therefore terminates PI3K signalling. Accumulation of PIP_3 on the cell membrane leads to the colocalization of signalling proteins with pleckstrin homology (PH) domains. This leads to the activation of these proteins and propagation of downstream PI3K signalling. Akt and phosphoinositide-dependent protein kinase 1 (PDK1) directly bind to PIP_3 and are thereby recruited to the plasma membrane. The phosphorylation of Akt at T308 (which is in the activation loop of Akt) by PDK1 and at S473 (which is in a hydrophobic motif of Akt) by mTOR complex 2 (mTORC2) results in full activation of this protein kinase[121]. In turn, Akt phosphorylates several cellular proteins, including glycogen synthase kinase 3α (GSK3α), GSK3β, forkhead box O transcription factors (FoxO), MDM2, BCL2-interacting mediator of cell death (BIM) and BCL2-associated agonist of cell death (BAD) to facilitate cell survival and cell cycle entry (for reviews, see REFS 3,6,122–124). In addition, Akt phosphorylates and inactivates tuberous sclerosis 2 (TSC2), a GTPase-activating protein for Ras homologue enriched in brain (RHEB)[125]. Inactivation of TSC2 allows RHEB to accumulate in the GTP-bound state and thereby activate mTORC1. The PI3K pathway through Akt regulates the use and uptake of glucose[114]. The therapeutic effects of perturbing cancer cell metabolism with PI3K pathway inhibitors remain largely unknown. Points of therapeutic inhibition are highlighted in the figure. Components in the signalling pathway that are mutated in cancers are shown in blue. AKTS1, AKT1 substrate 1 (also known as PRAS40); raptor, regulatory-associated protein of mTOR; rictor, rapamycin-insensitive companion of mTOR; SIN, stress-activated MAPK-interacting.

The structural differences between the helical and kinase domain mutants of PI3K were highlighted by a series of mutagenesis experiments which showed that kinase domain mutants, but not helical domain mutants, are still oncogenic when their Ras-binding domain is also mutated[42].

Recent data suggest that some cancers harbour activating mutations in the PI3K regulatory subunit, p85α (encoded by *PIK3R1*). The Cancer Genome Atlas Research Network identified *PIK3R1* mutations in 9 out of 91 human glioblastomas[43]. Interestingly, eight of these mutations were located in the inter-SH2 (iSH2) domain of p85α. In a previous study, mutations in the iSH2 domain were observed in 3 out of 80 ovarian cancers and 1 out of 60 colon carcinomas, and were shown to lead to constitutive PI3K–Akt signalling[44]. Structural data suggest that the iSH2 domain of the regulatory subunit interacts with the C2 domain of p110 (REF. 40). It seems

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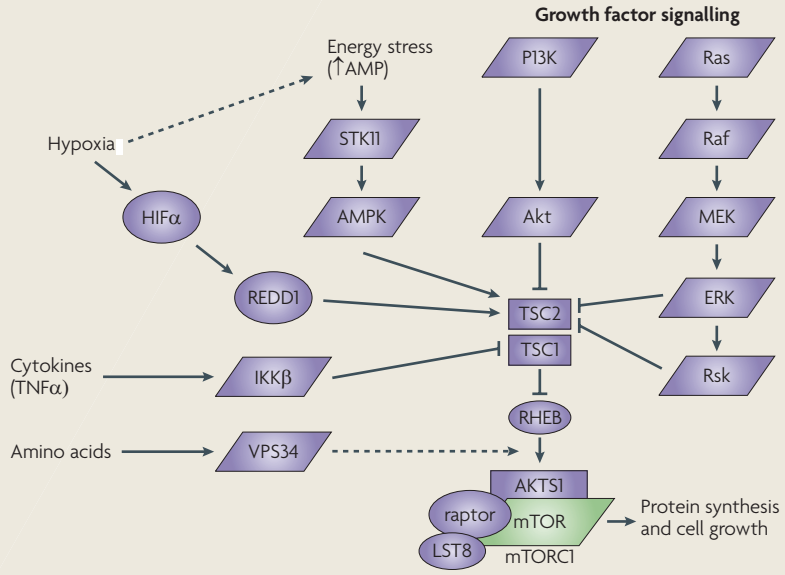
Box 2 | **mTOR complex 1 regulation**

mTOR is a serine–threonine kinase that is a member of the phosphatidylinositol kinase-related kinase (Pikk) family of kinases. mTOR exists in two distinct intracellular complexes, mTOR complex 1 and 2 (mTORC1 and mTORC2). As shown in the figure, mTORC1 is a complex of mTOR with regulatory-associated protein of mTOR (raptor), LST8 and AKT1 substrate 1 (AKTS1). Unlike mTORC2, mTORC1 is effectively inhibited by rapamycin and its analogues. mTORC1 phosphorylates p70 S6 kinase and 4E-binding protein 1 (4EBP1), 4EBP2 and 4EBP3. These phosphorylation events lead to the increased translation of mRNAs that encode many cell cycle regulators (such as MYC and cyclin D1), as well as certain ribosomal proteins and elongation factors (reviewed in REF. 69). mTORC1 activity is controlled by the tuberous sclerosis 1 (TSC1)–TSC2 complex. This complex functions as a GTPase-activating protein (GAP) for the small G protein Ras homologue enriched in brain (RHEB)[126–129]. In turn, GTP-bound RHEB directly activates mTORC1. To date, it seems that most inputs into mTORC1 regulation directly affect this TSC1–TSC2 complex, often by direct phosphorylation events. TSC2 is directly phosphorylated in response to growth factor signalling through PI3K–Akt and ERK–Rsk signalling and energy homeostasis through AMP-activated protein kinase (AMPK)[130]. Studies in *Drosophila melanogaster* and mammalian cells showed that Akt directly phosphorylates TSC2 (REFS 131,132), leading to decreased GAP activity of the complex, accumulation of RHEB-GTP and activation of mTORC1. However, it remains poorly understood how TSC2 phosphorylation by Akt leads to decreased GAP activity. Interestingly, hypoxia inhibits mTORC1 both by hindering ATP production (by activating the AMPK cascade) and by increasing expression of regulated in development and DNA damage response 1 (REDD1). Expression of REDD1 interferes with the ability of Akt to activate mTORC1 (REF. 133). Unlike the other modes of regulating mTORC1, it seems that amino acid availability might regulate mTORC1 through vacuolar protein sorting-associated 34 (VPS34) by a mechanism that is independent from the TSC1–TSC2 complex[134,135]. HIFα, hypoxia-inducible factor-α; IKKβ, inhibitor of nuclear factor-κB, subunit-β; STK11, serine–threonine kinase 11; TNFα, tumour necrosis factor-α.



likely that these mutations also activate PI3K by relieving the inhibitory effect of p85 on p110 (REFS 40,45). Notably, in the human glioblastoma samples used in these studies, the *PIK3CA* and *PIK3R1* mutations were mutually exclusive, suggesting a potential functional redundancy of these mutations as they both activate PI3K.

Genetic alterations of all three Akt isoforms have also been observed in cancers. Recently, a somatic mutation in *AKT1* was discovered in 8% (5 out of 81) of breast cancers, 6% (3 out of 51) of colorectal cancers and 2% (1 out of 50) of ovarian cancers[46]. This mutation, E17K, is in the pleckstrin homology (PH) domain and seems to allow promiscuous binding to the plasma membrane in the absence of 3′ phosphorylated phosphoinositides. Interestingly, this mutant showed constitutive phosphorylation of S473 in the absence of serum. By contrast, T308 phosphorylation remained responsive to serum, raising the possibility that cancers with this mutation might still require some PI3K activity for full activation of Akt. Interestingly, the E17K mutant displayed differential sensitivity to an Akt inhibitor that interacts with the PH domain. Therefore, there might be an opportunity to identify PH domain-binding inhibitors that preferentially inhibit the E17K mutant over wild-type Akt. Since the initial discovery of the E17K mutation in cancers, there have been other studies determining the frequency of this mutation in cancers. One study found this mutation in 4% (4 out of 93) of breast cancers, but it was not found in any non-small cell lung cancers ($n = 157$) or in acute myelogenous leukaemias ($n = 95$)[47]. Another study identified the E17K mutation in 5.6% (2 out of 36) of lung squamous cell cancers[48]. Recently, an identical mutation in *AKT3* was observed, albeit rarely, in melanoma samples and cell lines[49]. In addition, mutations in the AKT2 kinase domain were observed infrequently in colorectal cancers[50]. The presence of mutations in individual Akt isoforms suggests a potential role for Akt isoform-specific inhibitors in therapy (discussed below).

In addition to somatic mutations of *PTEN*, *PIK3CA*, *PIK3R1* and *Akt*, some cancers have amplifications of *AKT1*, *AKT2* and *PIK3CA* (TABLE 1) (reviewed in REFS 3,51). However, it is not well understood how these amplifications qualitatively or quantitatively affect PI3K signalling and whether they obviate the usual mechanisms for activating PI3K.

Implications for isoform-specific inhibitors

Many of the PI3K inhibitors that are currently in clinical development inhibit all of the catalytic subunit isoforms of class I_A PI3Ks, p110α, p110β and p110δ, whereas others only inhibit individual isoforms. However, it remains unclear which type of inhibitor will be more effective clinically, isoform-specific inhibitors or pan-PI3K inhibitors. Similarly, it is unknown whether there will be an advantage to isoform-specific Akt inhibitors. The answer partly depends on the additional toxicity caused by complete inhibition of all isoforms when using non-selective inhibitors, and on whether one can identify cancers in which inhibition of only one isoform will be sufficient to turn off PI3K–Akt signalling. Interestingly, recent studies suggest that transient complete inhibition of a target kinase might be more crucial than chronic incomplete inhibition[52]. This raises the concern that if complete inhibition of all PI3K or Akt isoforms is too toxic to patients, non-selective inhibitors might be tolerable only at doses that cause less than 100% inhibition of all PI3K or Akt isoforms.

p110. In normal tissues, p110α and p110β are ubiquitously expressed, whereas the expression of p110δ and p110γ is mostly restricted to leukocytes. Accordingly, mice with genetic loss of either p110α or p110β die during early

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Table 1 | **Somatic genetic mutations activating the PI3K-Akt pathway**

Genetic change	Most common cancer types	Refs
Genetic mutation		
PTEN	Endometrial, glioblastoma, melanoma, prostate, breast, ovarian	*
PIK3CA (p110α)	Breast, colon, endometrial, glioblastoma, ovarian	*
AKT1 (E17K)	Breast, colorectal, squamous cell lung carcinoma	46–48
AKT2	Colorectal	50
AKT3 (E17K)	Melanoma	49
PIK3R1 (p85α)	Glioblastoma, ovarian	43,44
Genetic amplification		
PIK3CA	Head and neck, squamous cell lung carcinoma, cervical, gastric, oesophageal	138–144
AKT1	Gastric	145
AKT2	Head and neck, pancreatic, ovary, breast	138, 146–148

*Catalogue of Somatic Mutations in Cancer (see Further information).

embryogenesis[53,54], whereas mice with loss of p110δ and p110γ are viable but immune deficient[55–59]. The somatic mutations found in p110α underscore its prominent role in PI3K signalling in cancer (discussed above). However, the contributions of other p110 isoforms remain less well understood.

A potential specific role for p110β in cancer might also be emerging, especially in *PTEN*-deficient cancers. Zhao and colleagues used genetically engineered mice that develop *Pten*-deficient prostate intraepithelial neoplasia to assess the relative importance of p110α and p110β[60]. Interestingly, they observed that loss of p110β, but not p110α, obliterated prostate intraepithelical neoplasia formation. Another study showed that knockdown of p110β with short-hairpin RNAs effectively downregulated PI3K–Akt signalling in three different *PTEN*-deficient cancer cell lines, and that restoration of PI3K–Akt signalling required intact lipid kinase activity[61]. By contrast, knockdown of p110α expression did not inhibit PI3K signalling in *PTEN*-deficient cancers even though p110α knockdown produced dramatic effects in cancers with *PIK3CA* mutations. Furthermore, another study reported that p110β knockdown, but not p110α knockdown, blocked androgen receptor transactivation in *PTEN*-deficient LnCAP prostate cancer cells[62]. Although preliminary, these studies suggest that p110β isoform-specific inhibitors might effectively downregulate PI3K–Akt signalling in some *PTEN*-deficient cancers.

However, it should be noted that gene deletion and knockdown studies might not necessarily reflect chemical inhibition, as there might be kinase-independent functions of p110β that are required for tumorigenesis in *PTEN*-deficient cancers. Interestingly, the potential differences between gene deletion studies and chemical inhibition studies were underscored by a comparison of p110γ-knockout and p110γ catalytically inactive knock-in mice[63,64]. Although both mice had similar levels of immune cell dysfunction, they had different cardiac phenotypes. The cardiac defect in the p110γ-knockout mice was caused by the loss of the scaffolding function of p110γ; however, this function was retained in the knock-in mice that expressed a catalytically inactive form of p110γ. These studies show the potential limitations of extrapolating the effects of gene knockout or knockdown studies to the effects of chemical inhibition.

Akt. There is increasing evidence that the different Akt isoforms have non-overlapping functions in cancer. For example, AKT2 overexpression was commonly observed in late-stage colorectal cancers and metastases[65]. Loss of AKT2 expression inhibited the metastatic potential of colorectal cancer cell lines, and this phenotype was not restored by AKT1 overexpression. Furthermore, studies in both mammary epithelial cell lines and in genetically engineered mouse breast cancer models suggest that AKT2 promotes cellular invasiveness and mesenchymal characteristics, whereas AKT1 promotes cellular survival and growth[66,67]. Interestingly, loss of AKT1 promoted cellular invasiveness and metastasis, presumably by shifting the balance of signalling through AKT2. These results raise concerns about potential deleterious effects that could result from specific inhibition of AKT1.

Inhibitors

Several small molecules that inhibit the PI3K–Akt signalling pathway are in clinical development. For the purposes of this Review, four main classes of inhibitors are discussed: dual PI3K–mTOR inhibitors, PI3K inhibitors, Akt inhibitors and mTOR inhibitors (TABLE 2). Rapamycin and its analogues that specifically inhibit the mTORC1 complex will not be discussed as they have been the subjects of many recent reviews[68–72]. The mTOR inhibitors discussed here are catalytic site inhibitors that inhibit both mTORC1 and mTORC2.

Dual PI3K–mTOR inhibitors. The p110 subunits of PI3K and mTOR share similar structures, and small molecule inhibitors of p110 often also inhibit mTOR[7]. Most of the dual PI3K–mTOR inhibitors target the p110α, β and δ isoforms, mTORC1 and mTORC2. The potential advantages for this class of drugs are straightforward as complete inhibition of all the p110 isoforms as well as mTORC1 and mTORC2 would be expected to effectively

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Table 2 | **PI3K–Akt pathway inhibitors in clinical development for treating cancers**

Inhibitor	Company	Phase of clinical trial	Refs
Dual PI3K and mTOR inhibitors			
BEZ235	Novartis	Phase I/II	37,92,96,103,149
BGT226	Novartis	Phase I/II	NS
XL765	Exelixis	Phase I	NS
SF1126	Semafore	Phase I/II	NS
GSK1059615	GSK	Preclinical	150
PI3K inhibitors			
XL147	Exelixis	Phase I	NS
PX866	Oncothyreon	Phase I	100,151,152
GDC0941	Genentech/Piramed/Roche	Phase I	NS
BKM120	Novartis	Phase I	NS
CAL101 (targets p110δ)	Calistoga Pharmaceuticals	Phase I	NS
Akt inhibitors			
Perifosine	Keryx	Phase I/II	153–156
GSK690693	GSK	Phase I	157,158
VQD002	Vioquest	Phase I	NS
MK2206	Merck	Phase I	NS
mTOR inhibitors (catalytic site)			
OSI027	OSI Pharmaceuticals	Phase I	NS
AZD8055	AstraZeneca	Phase I/II	NS

NS, not stated.

shut down PI3K–Akt–mTORC1 signalling (BOX 1). The dual PI3K–mTOR inhibitors should remain effective in some cancers that can circumvent other PI3K and Akt inhibitors (see below). A key issue that will influence the advantage of dual PI3K–mTOR inhibitors is whether the complete inhibition of all p110 isoforms, mTORC1 and mTORC2 will be tolerable in patients or whether the use of these inhibitors will necessitate sacrificing the complete inhibition of one or more of the potential targets. It is expected that these inhibitors will effectively shut down PI3K–Akt signalling in cancers with *PIK3CA* mutations, *PIK3R1* mutations, PTEN loss and RTK-dependent activation. This class of inhibitors might even be effective in cancers with Akt mutations or amplifications, as both PI3K and mTORC2 activity might be required for full Akt activation even in these settings (BOX 1). In addition, it is well established that mTORC1 inhibitors (that is, rapamycin and its analogues) often lead to a feedback activation of PI3K in cancers[73]. Dual PI3K–mTOR inhibitors might therefore mitigate this feedback activation of PI3K signalling and yield greater therapeutic benefit[74].

PI3K inhibitors. The PI3K inhibitors can be divided into isoform-specific inhibitors or pan-PI3K inhibitors. The pan-PI3K inhibitors target all class I_A PI3Ks in the tumour. However, a theoretical advantage of isoform-specific inhibitors is that they might be tolerated at doses that result in complete target inhibition without producing untoward side effects, such as immunosuppression and glucose intolerance. Indeed, isoform-specific inhibitors might be particularly effective in certain cancers; for example, p110α-specific inhibitors might effectively shut off PI3K–Akt signalling in cancers with *PIK3CA* mutations. In addition, recent data suggest that p110α might be the predominant catalytic isoform in vasculogenesis, and that specific p110α inhibitors might block angiogenesis[75]. Furthermore, a preliminary study that compared isoform-selective PI3K inhibitors suggests that p110α might be the crucial PI3K isoform in breast cancers with *ERBB2* amplifications[76]. Using RNA interference, one group found that silencing p110α, but not p110β or p110δ, led to decreased growth and increased apoptosis of medulloblastoma cells[77]. In addition to cancers that might be effectively treated with p110α-specific inhibitors, some cancers might benefit from p110β-specific inhibitors. As mentioned above, recent studies have suggested a prominent role for p110β, but not p110α, in PI3K signalling in some PTEN-deficient cancers. Indeed, these preclinical studies suggest that p110β-specific inhibitors might be effective for this subset of cancers[60,61,76]. However, even in cancers that seem to be specifically reliant on either p110α or p110β, there is the concern that other non-targeted p110 isoforms might eventually compensate for decreased activity of the targeted isoform. On a cautionary note, the reliance on p110α or p110β isoform-specific inhibitors might ignore the role of the p110δ isoform in human solid and haematological malignancies[78–82]. Indeed, encouraging results using a p110δ-specific inhibitor for refractory non-Hodgkin's lymphoma and chronic lymphocytic leukaemia were recently presented[83].

Vasculogenesis
The process of blood vessel formation that occurs by the production of endothelial cells.

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There might be substantial differences between the efficacies of PI3K inhibitors and dual PI3K–mTOR inhibitors, depending on whether PI3K inhibition alone leads to loss of mTORC1 signalling in the particular cancer that is being treated. Surprisingly, the common assumption that PI3K inhibition alone will lead to mTORC1 inhibition in most cancers remains untested in the laboratory, as many compounds used experimentally inhibit both PI3K and mTOR[84]. A fair prediction is that there are some cancers in which PI3K–Akt is the strongest input for mTORC1 signalling, such as tumours with *PIK3CA* mutations or loss of PTEN. In these cases, it might be advantageous to use specific PI3K inhibitors, which would effectively downregulate mTORC1 signalling and avoid toxicities from the effects of direct mTORC1 and mTORC2 inhibition in non-cancerous cells. Indeed, the combination of specific PI3K inhibitors and other pathway inhibitors, such as MEK inhibitors, might be better tolerated than dual PI3K–mTOR inhibitors (see below). However, there are also cancers in which PI3K–Akt signalling does not solely control mTORC1 activity — such as cancers with *BRAF* or *KRAS* mutations — which might benefit from dual PI3K–mTOR inhibitors (J.A.E. and H. Ebi, unpublished observations). Notably, a disadvantage of PI3K inhibitors is that they might not effectively downregulate Akt activation in cancers with AKT1-E17K mutations or *AKT1* or *AKT2* amplifications (as these inhibitors do not inhibit mTORC2).

Akt inhibitors. Several companies are targeting Akt with both ATP mimetics and non-catalytic site inhibitors[85]. Cancers with *AKT1* mutations and *AKT1* and *AKT2* amplifications might be expected to be more sensitive to Akt inhibitors. The type of Akt inhibitor, ATP mimetic or allosteric, will affect the pharmacodynamic analyses that are used to assess target inhibition. As allosteric Akt inhibitors block the recruitment of Akt to the membrane by interfering with the binding of the PH domain to phosphoinositides, loss of Akt phosphorylation serves as a pharmacodynamic measure of target inhibition[85]. By contrast, Akt catalytic site inhibitors might not block Akt phosphorylation, and might increase its phosphorylation through loss of negative-feedback regulation of PI3K[86]. Therefore, for catalytic site inhibitors, one will need to assess the phosphorylation status of Akt substrates, such as AKT1 substrate 1 (AKTS1; also known as PRAS40), glycogen synthase kinase 3 (GSK3) and forkhead box transcription factors (BOX 1).

The distinct functions of AKT1 and AKT2 in cancers might spur the development of isoform-specific Akt inhibitors. AKT1 is linked to cell survival and growth, whereas AKT2 is linked to invasiveness. In addition, mouse and human studies suggest a prominent role for AKT2 in insulin responsiveness, and the loss of AKT2 activity might promote a strong diabetic phenotype[87,88]. If toxicity is dose limiting in clinical development, it will be important to determine if isoform-specific inhibitors are better tolerated than pan-Akt inhibitors.

Importantly, the careful comparison of PI3K and Akt inhibitors might lead to the identification of important non-Akt effectors of the PI3K pathway. The BTK family of non-RTKs and the SGKs have been implicated as effectors of pro-survival and pro-growth signalling from PI3Ks[8]. Recently, Akt was shown to be a less crucial effector of cell survival than SGK3 in a subset of cancers with *PIK3CA* mutations[159]. The prevalence and importance of these Akt-independent effectors of PI3K signalling might substantially affect the clinical effectiveness of Akt inhibitors.

mTOR catalytic site inhibitors. mTOR catalytic site inhibitors directly inhibit mTOR and they are therefore expected to inhibit both the mTORC1 and mTORC2 complexes. These compounds should have the same effect as rapamycin (that is, mTORC1 inhibition) and inhibit Akt S473 phosphorylation (that is, mTORC2 inhibition) (BOX 1). Interestingly, two independent studies observed that mTOR catalytic site inhibitors were more potent than rapamycin in their inhibition of mTORC1 and this was likely to account for their increased anti-proliferative activity compared with rapamycin[89,90]. Although the concomitant inhibition of Akt S473 phosphorylation might mitigate the feedback activation of the PI3K pathway that is induced by mTORC1 inhibition[73], these compounds might not block T308 phosphorylation[73]. This is concerning because previous studies have suggested that loss of S473 phosphorylation might disable some, but not all, components of Akt signalling[91]. Indeed, in one study, an mTOR catalytic site inhibitor had minimal effects on the phosphorylation state of several Akt substrates despite effectively inhibiting Akt S473 phosphorylation[90]. The activity of these compounds might therefore be more closely related to their complete inhibition of mTORC1 rather than their effects on Akt phosphorylation. Finally, feedback activation of PI3K from mTORC1 inhibition might result in the hyperactivation of Akt-independent effectors of PI3K signalling.

Preclinical studies

Although PI3K pathway inhibitors are just entering the clinic, there are emerging preclinical studies that suggest how they should be most appropriately used. For example, there have been reports suggesting that breast cancers possessing *PIK3CA* mutations might be among the more sensitive cancers to single-agent Akt and dual PI3K–mTOR inhibitors[85,92]. However, even in these sensitive preclinical models, these drugs seem to primarily promote tumour growth stasis or delay tumour growth *in vivo*, as substantial tumour shrinkage was not observed[85,92]. Moreover, there is a growing number of preclinical cancer models that fail to show any induction of apoptosis despite effective PI3K–Akt inhibition and cytostasis[74,93]. If extrapolated to clinical trials, these data suggest that patients might benefit and show stable disease and longer times to progression, but responses to therapy that meet RECIST criteria might be less common. Therefore, in contrast to the effects of targeted therapies in some other cancer paradigms that show oncogene addiction (for example, EGFR inhibitors in *EGFR*-mutant lung cancers),

Pharmacodynamic
The study of the biochemical and physiological effects of drugs on the body, the mechanisms of drug action and the relationship between drug concentration and effect.

RECIST
RECIST (Response Evaluation Criteria In Solid Tumours) is a set of published rules that define when cancer patients improve, stay the same or progress during treatments.

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single-agent PI3K pathway inhibitors might not typically yield such dramatic responses in cancers that are sensitive to these inhibitors. Unlike breast cancers with *PIK3CA* mutations, murine lung adenocarcinomas induced by a transgene expressing the p110α-H1047R mutant were highly sensitive to a dual PI3K–mTOR inhibitor and displayed dramatic tumour shrinkage[37]. These differences between human cancers and animal models might underscore the greater biological complexity of human cancer cell lines compared with a transgenic animal in which tumorigenesis is primarily driven by a single oncogene.

It is unknown whether other cancer types with *PIK3CA* mutations will be sensitive to PI3K inhibitors. Notably, colorectal cancers have a high prevalence of *PIK3CA* mutations (~25%). However, these cancers often have concurrent mutations in *KRAS*[94,95], and one might predict that the presence of *KRAS* mutations might adversely impact the effectiveness of single-agent PI3K pathway inhibitors (see below).

In addition to breast cancers with *PIK3CA* mutations, PI3K pathway inhibitors seem to have single-agent activity in breast cancers with *ERBB2* amplifications[85,92]. This suggests that these cancers are particularly reliant on the PI3K signalling pathway. Indeed, when breast cancers with *ERBB2* amplifications become resistant to anti-ERBB2 therapies, they still seem to require PI3K signalling for growth and survival[96]. Therefore, there is enthusiasm for the development of these agents for cancers that have developed resistance to therapies that target ERBB2. In addition, cancers with *ERBB2* amplifications that are treated with trastuzumab have an inferior prognosis when they harbour coexisting *PIK3CA* mutations or have lost PTEN[17]. This suggests that combining PI3K pathway inhibitors with anti-ERBB2 therapies will also be potentially beneficial in these settings.

Interestingly, in lung cancers, the regulation of PI3K signalling affects their susceptibility to EGFR inhibitors. In lung cancers that are sensitive to EGFR inhibitors, both PI3K and ERK signalling are under the sole control of EGFR. After treatment with EGFR inhibitors, both pathways shut down and the lung cancer cells undergo apoptosis (FIG. 1). These cancers can be rendered resistant to EGFR inhibitors simply by maintaining PI3K signalling, and reactivation of PI3K signalling is almost invariably observed in cancers that naturally develop acquired resistance to EGFR inhibitors[19,20,97–99] (FIG. 1). Some mechanisms of acquired resistance to EGFR inhibitors, such as secondary mutations in *EGFR* (T790M) and amplification of the *MET* oncogene, led to persistent activation of multiple downstream signalling pathways despite EGFR inhibition[19,97]. In other resistance models, PI3K signalling is reactivated and ERK phosphorylation remains suppressed[20]. This was observed in a model of resistance that was driven by activation of the insulin-like growth factor receptor (IGF-IR)–PI3K–Akt signalling axis. However, the resistant cells were sensitive to either IGF-IR or PI3K inhibitors when combined with EGFR inhibitors. Findings such as this have spurred the development of therapies that combine PI3K pathway inhibitors with EGFR inhibitors, both to increase the proportion of cancers that benefit from EGFR inhibitors and to delay the development of resistance in those cancers that are initially responsive.

Although the identification of biomarkers that predict sensitivity to PI3K pathway inhibitors will be important, biomarkers that predict resistance can also be useful. There are emerging data suggesting that cancers with *KRAS* mutations are unlikely to be sensitive to single-agent PI3K inhibitors[37,76,100]. This was initially surprising as previous studies have shown that PI3K has an important role in KRAS-induced lung tumorigenesis[101,102]. However, blocking tumorigenesis and decreasing the size of established tumours are distinct outcomes, and we and others have failed to observe potent anti-tumour activity using single-agent PI3K pathway inhibitors in cancers with *KRAS* mutations[37,76,100]. As discussed below, inhibitors of the PI3K pathway might be effective in cancers that have *KRAS* mutations when they are combined with therapies that target additional pathways.

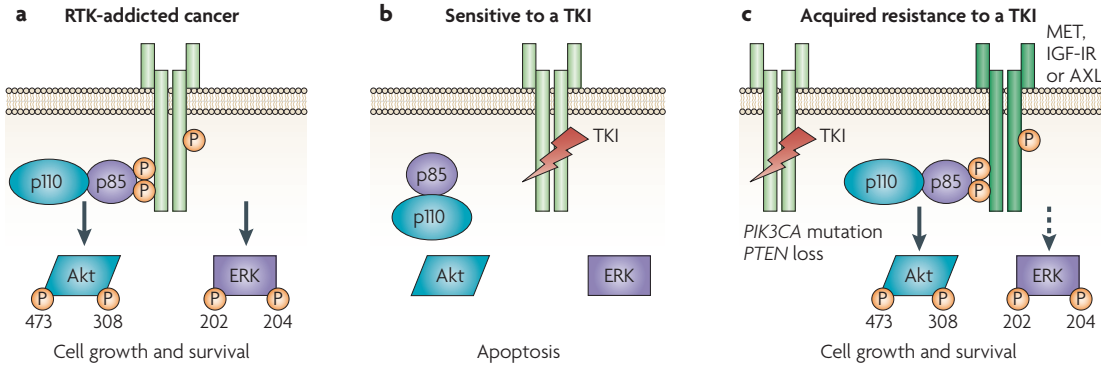

Figure 1 | **Mechanisms of acquired resistance to receptor tyrosine kinase inhibitors. a** | Cancers that are addicted to receptor tyrosine kinases (RTKs) have PI3K signalling and ERK activation that is under the sole control of the RTK. **b** | In cancers that are sensitive to tyrosine kinase inhibitors (TKIs), inhibition of the RTK leads to loss of PI3K and ERK signalling. **c** | Cancers become resistant when the cancer finds other means to activate downstream signalling, especially PI3K signalling (reviewed in REFS 136,137). This resistance has been shown to occur with *MET* amplification, insulin-like growth factor 1 receptor (IGF-IR) activation and AXL activation. In addition, loss of PTEN and the presence of *PIK3CA* (which encodes the p110α subunit of PI3K) mutations are associated with resistance to RTKs.

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Although much of the preclinical work has assessed the potential therapeutic benefits from inhibiting the PI3K signalling pathway in cancer cells, there might be potential therapeutic benefits from inhibiting PI3K signalling in the cancer microenvironment. Indeed, recent preclinical studies have shown that p110α is crucial for angiogenesis[75], and that PI3K pathway inhibitors might impair cancer-induced angiogenesis and affect vessel permeability[103,104]. Furthermore, the effects of PI3K pathway inhibitors on cancer-related inflammation and stromal cells remain unknown.

Clinical development of PI3K pathway inhibitors

Given the large number of PI3K pathway therapeutics that are in development (TABLE 2) and the many potential clinical scenarios for their use, thoughtful and efficient drug development strategies are needed. In general, there seem to be two main strategies emerging. One is to test these compounds in a broad range of cancers to identify those in which the compounds work, and the second strategy is to use the most compelling preclinical data to guide genotype-directed trials (such as using PI3K pathway inhibitors in breast cancers with *PIK3CA* mutations or *ERBB2* amplifications). Arguably, if a drug fails to show activity in these 'sensitive' cancers, there is little chance that it will be beneficial as a single agent in other cancer types. Such genotype-directed clinical trials will facilitate indirect comparisons of the different PI3K pathway inhibitors and prioritize the most active therapies. However, preclinical data might be insufficient or incomplete for accurate guidance of patient selection. For example, when EGFR tyrosine kinase inhibitors were developed for lung cancers, many believed that they would work best in squamous cell carcinomas, as these cancers have the highest expression of EGFR and the A431 squamous carcinoma cell line was one of the most sensitive preclinical models. Only after observing the activities of EGFR inhibitors in large clinical trials of unselected patients, was it discovered that a subset of lung cancers harbour mutations in *EGFR* and that these cancers were the most sensitive to EGFR inhibitors[105–107]. Although this example might seem less likely with the current level of preclinical analyses, it does highlight the probability that our knowledge will be more impressive in hindsight. We will therefore need to continually re-evaluate preclinical models in light of the results from clinical trials.

To effectively develop PI3K pathway inhibitors, it will be helpful to understand why these compounds fail when they do. Are they unable to potently inhibit the target *in vivo* or is effective target inhibition insufficient to produce the desired clinical benefit? Pharmacodynamic assessments of target inhibition will therefore be paramount. Such analyses require the assessment of cancer specimens after drug treatment. Although biomarkers of the PI3K pathway can be measured by immunohistochemical (IHC) analyses using phospho-specific antibodies, routine IHC is largely qualitative and more quantitative analyses will be needed. Technologies such as reverse-phase protein arrays that were developed by Mills and colleagues[108] and highly sensitive mass spectrometric assays[109] might ultimately complement IHC to quantitatively assess pathway activation. Although obtaining cancer specimens after treatment is possible, it is not standard practice and requires commitment from investigators, caregivers and patients. Some investigators have taken advantage of the neoadjuvant setting to assess target inhibition before surgical resection[110,111]. However, these studies can be limited by the amount of time that is required to elapse between the last neoadjuvant treatment and surgical resection. Although pharmacodynamic studies are more costly and potentially more invasive, a thorough study of 20–30 patients might save enormous amounts of time and resources. Importantly, these studies might prevent future patients from enrolling in trials with drugs that fail to effectively inhibit their targets *in vivo*.

The hurdles involved in tissue acquisition before and after drug treatments underscore the need to develop less invasive ways to measure target inhibition. One promising approach might be the assessment of circulating tumour cells[112,113]. Rare cancer cells circulate in the blood and can be isolated from routine blood samples using antibody-based techniques. Although circulating tumour cells have been used to evaluate the expression of tumour markers and to identify cancer-associated genetic mutations, one can envision using these cells for pharmacodynamic analyses of cell signalling. These analyses could be performed either by IHC or perhaps by fluorescence-activated cell sorting. However, it remains unknown if assessment of circulating tumour cells will accurately reflect the effect of a drug on cancers *in situ* owing to differences in drug delivery and cancer biology.

In addition, imaging techniques might be developed to evaluate the activity of PI3K pathway inhibitors in patients. Although there are currently no available imaging techniques that directly assess PI3K signalling activity, FDG-PET ([^{18}F] 2-fluoro-2-deoxy-D-glucose positron emission tomography) imaging might be useful. The PI3K pathway regulates glucose uptake and metabolism, and cancers with high levels of PI3K signalling might require high rates of glycolysis for their survival[114]. Indeed, prostate tumours that are induced by activated Akt stimulate glycolysis in an mTORC1-dependent manner[115]. We recently observed that NVP-BEZ235, a dual PI3K–mTOR inhibitor, led to the rapid resolution of FDG avidity in mouse lung adenocarcinomas driven by the p110α-H1047R mutant[37]. Notably, when the same drug was administered to a lung cancer model induced by oncogenic *KRAS*, we did not observe a change in FDG avidity and the drug was ineffective despite inhibition of the PI3K–Akt pathway[37]. A change in FDG avidity might therefore be a rapid marker of efficacy for PI3K pathway inhibitors, but it remains unclear whether it will be a good biomarker for effective PI3K pathway inhibition in cancers that are unresponsive to therapy.

Interestingly, insulin resistance (which is manifested as hyperinsulinaemia or hyperglycaemia), a potential toxicity caused by on-target effects of PI3K inhibitors, might be an effective pharmacodynamic marker for PI3K pathway inhibition in liver, fat and muscle. However, this biomarker will not necessarily reflect target inhibition in cancers. In addition, all types of PI3K pathway inhibitors will not

Neoadjuvant
A cancer therapy that is delivered before a surgical procedure.

Insulin resistance
The condition in which normal amounts of insulin are inadequate for the production of a normal insulin response from fat, muscle and liver cells.

Hyperinsulinaemia
Increased insulin levels in the blood. This is often observed when a person becomes insulin resistant as their body attempts to control blood glucose levels.

Hyperglycaemia
Increased levels of glucose in the blood. This usually occurs in adult-onset diabetes because insulin-sensitive tissues become less responsive to insulin.

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promote insulin resistance at equal levels. For example, PI3K inhibitors might not promote as much insulin resistance as Akt inhibitors. Indeed, a study showed that liver-specific knockout of *Pik3r1* on a heterozygous *Pik3r2* (which encodes p85β) background did not compromise Akt activation by insulin, although p110 levels were reduced by 90%[116]. A plausible explanation is that insulin normally induces PIP_3 production in the liver in a large excess over the levels that are needed to activate Akt. When PI3K is inhibited, it might not affect Akt activation unless PI3K inhibition is 95% or more (L. Cantley, personal communication). A recent study showed that the peroxisome proliferator-activated receptor-γ agonist pioglitazone could overcome the glucose intolerance induced by the PI3K inhibitor PX866 (REF. 117).

Anticipated therapeutic limitations. Will PI3K pathway inhibitors be effective single-agent cancer therapeutics? Most, but not all, successful targeted therapies in the clinic have been primarily directed against tyrosine kinases, such as BCR–ABL, KIT, EGFR and ERBB2. In these cases, target inhibition leads to downregulation of multiple intracellular pathways, not just PI3K. It remains unclear whether downregulation of PI3K alone will recapitulate some of these previous successes.

One potential reason for the limited efficacy of single-agent PI3K pathway inhibitors is the presence of signalling feedback loops in cells. Inhibition of PI3K might alleviate the repression of other pro-survival and growth pathways. For example, mTORC1 inhibition leads to activation of PI3K signalling through a feedback loop[73], and this has been proposed as a possible reason for the limited efficacy of rapamycin in epithelial cancers[73]. It is therefore likely that mTOR and Akt inhibitors will increase PI3K activity and increase signalling along Akt-independent arms of the PI3K signalling pathway. More recently, studies have shown that inhibition of mTORC1 also leads to activation of the ERK signalling pathway[118], raising concerns that this feedback might also mitigate the effectiveness of PI3K pathway inhibitors.

These studies suggest that combining PI3K pathway inhibitors with other therapies might improve efficacy. When tyrosine kinase inhibitors are effective, they lead to downregulation of both the PI3K and ERK pathways. There is growing evidence that inhibition of both pathways might be substantially more effective than inhibition of either pathway alone[37,119]. In addition to their divergent signalling, the PI3K and ERK pathways also converge on the BH3 family of proteins, which regulate apoptosis, and the mTORC1 signalling pathway, which regulates cell growth (FIG. 2). We recently examined the efficacy of combining PI3K and MEK inhibitors in murine models of lung cancers that expressed mutant *Kras*[37]. Although neither inhibitor alone had a substantial effect on these cancers, their combination was highly effective. Importantly, these studies showed that the combination of a PI3K and MEK inhibitor is not toxic to mice, and in turn, increases the rationale for combining these therapies in patients.

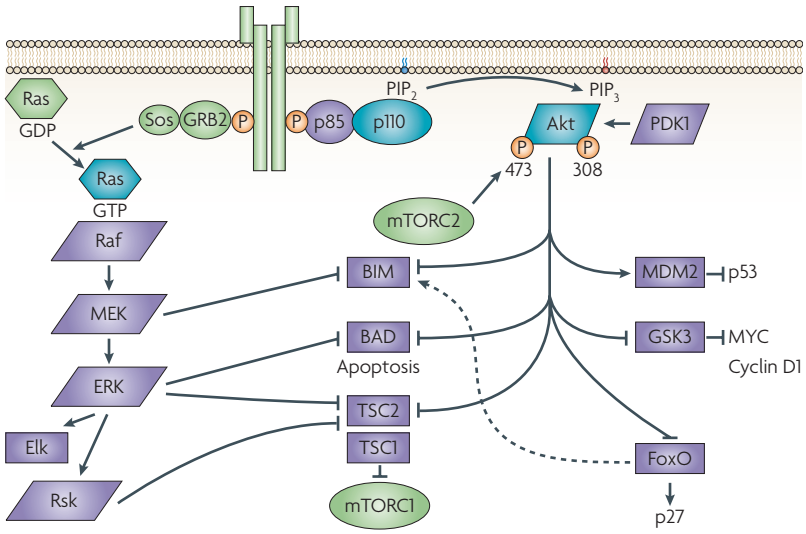

Figure 2 | **Combined PI3K and MEK pathway inhibition.** The PI3K–Akt signalling pathway and the Raf–MEK–ERK pathway each promote cell growth and survival. Each pathway has its own distinct downstream effects. However, in addition to their divergent paths, these two pathways converge on at least two crucial downstream targets, mTOR complex 1 (mTORC1) and the BH3 family of proteins that regulate apoptosis. For many cancers, combined PI3K–Akt and Raf–MEK–ERK inhibition might be required to effectively shut off mTORC1 signalling and promote apoptosis through the BH3 family of proteins. When Ras (KRAS or NRAS) is activated by mutation (shown in blue), inhibition might require combining inhibitors of both the PI3K and MEK pathways[37]. BAD, BCL2-associated agonist of cell death; BIM, BCL2-interacting mediator of cell death; FoxO, forkhead box O; GRB2, growth factor receptor-bound 2; GSK3, glycogen synthase kinase 3; PDK1, phosphoinositide-dependent protein kinase 1; PIP_2, phosphatidylinositol-4, 5-bisphosphate; PIP_3, phosphatidylinositol-3, 4, 5-trisphosphate; Sos, son of sevenless; TSC, tuberous sclerosis complex.

Conclusions

Although the excitement regarding the potential benefits of inhibiting the PI3K pathway alone or in combination with the ERK pathway seems well founded, and it is possible that these treatments will yield meaningful benefits for cancer patients, it seems safe to assume that neither of these treatment strategies will produce many cures for patients with advanced cancer. Moreover, resistance is likely to emerge, as we have observed in preclinical models (J.A.E., unpublished observations). Indeed, it is possible that the drug targets will become mutated and resistant to particular inhibitors. For example, Shokat and colleagues have already identified mutations in p110α that confer resistance to some inhibitors[120]. Therefore, even after we discover how to shut down these pathways and produce clinical benefits, we will have to identify other complementary therapies to overcome resistance and combat the ongoing adaptation of cancers.

In conclusion, we are embarking on an exciting journey. All the genetic and laboratory studies that have been performed over the past 20 years are culminating in clinical trials that examine PI3K–Akt pathway inhibitors as cancer therapeutics. As these compounds move into the clinic, laboratory efforts will need to intensify to study the unexpected observations that will invariably emanate from the clinic. When the efficacies of these compounds are different from what we had anticipated, it is imperative that we determine the reasons for these discrepancies. Such investigations should spur the development of improved therapeutic strategies and allow us to realize their ultimate potential.

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Acknowledgements
I am grateful to L. Cantley and the members of his laboratory for discussions and insights regarding PI3K signalling. I thank my laboratory, P. Jänne's laboratory and J. Settleman's laboratory for discussions regarding oncogene addiction and resistance to targeted therapies. I apologize to the many authors whose work I could not cite directly because of space limitations. This work was supported by the National Institutes of Health.

Competing interests statement
The author declares competing financial interests. See web version for details.

DATABASES.
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
AKT1 | *AKT3* | *BRAF* | *EGFR* | *ERBB2* | *KRAS* | *MET* | *PIK3CA* | *PIK3R1* | *Pik3r2* | *PTEN*
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary
[^{18}F] 2-fluoro-2-deoxy-D-glucose | erlotinib | gefitinib | imatinib | pioglitazone | PX866 | rapamycin | trastuzumab
UniProtKB: http://www.uniprot.org
AKTS1 | AKT2 | BTK | IGF-IR | KIT | mTOR

FURTHER INFORMATION
Jeffrey Engelman's homepage: http://www2.massgeneral.org/cancer-research/groups.aspx?member=879
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