PI3K and cancer: lessons, challenges and opportunities

David A. Fruman[1] and Christian Rommel[2]

Abstract | The central role of phosphoinositide 3-kinase (PI3K) activation in tumour cell biology has prompted a sizeable effort to target PI3K and/or downstream kinases such as AKT and mammalian target of rapamycin (mTOR) in cancer. However, emerging clinical data show limited single-agent activity of inhibitors targeting PI3K, AKT or mTOR at tolerated doses. One exception is the response to PI3Kδ inhibitors in chronic lymphocytic leukaemia, where a combination of cell-intrinsic and -extrinsic activities drive efficacy. Here, we review key challenges and opportunities for the clinical development of inhibitors targeting the PI3K–AKT–mTOR pathway. Through a greater focus on patient selection, increased understanding of immune modulation and strategic application of rational combinations, it should be possible to realize the potential of this promising class of targeted anticancer agents.

Phosphoinositide 3-kinase (PI3K). A lipid kinase that produces phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3; also known as PIP_3), which is a key signalling lipid.

AKT
A serine/threonine kinase whose activation is dependent on phosphoinositide 3-kinase.

Mammalian target of rapamycin (mTOR). A serine/threonine kinase that functions in two distinct complexes: mTOR complex 1 (mTORC1) and mTORC2.

[1]*Department of Molecular Biology & Biochemistry, and Institute for Immunology, University of California, Irvine, 3242 McGaugh Hall, Irvine, California 92697, USA.*
[2]*Amgen Inc., Thousand Oaks, One Amgen Center Drive, California 91320, USA.*
e-mails: dfruman@uci.edu; crommel@amgen.com
doi:10.1038/nrd4204

The signalling network defined by phosphoinositide 3-kinase (PI3K), AKT and mammalian target of rapamycin (mTOR) controls most hallmarks of cancer, including cell cycle, survival, metabolism, motility and genomic instability[1]. The pathway also contributes to cancer-promoting aspects of the tumour environment, such as angiogenesis and inflammatory cell recruitment[2–4] (FIG. 1). The lipid second messenger produced by PI3K enzymes, phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3; also known as PIP_3), is constitutively elevated in most cancer cells and recruits cytoplasmic proteins to membrane-localized 'onco' signalosomes[5,6]. The oncogenic signalling proteins recruited in this way include members of the AGC kinase family (for example, AKT) (FIG. 1), TEC family tyrosine kinases and various modulators of small GTPase activity[7].

Cancer genetic studies suggest that the PI3K pathway is the most frequently altered pathway in human tumours: the *PIK3CA* gene (encoding the PI3K catalytic isoform p110α) is the second most frequently mutated oncogene, and *PTEN* (encoding phosphatase and tensin homolog, the major PtdIns(3,4,5)P_3 phosphatase) is among the most frequently mutated tumour suppressor genes[8,9]. In accord, a recent genomic study of head and neck cancer found the PI3K pathway to be the most frequently mutated[10]. Indeed, even in cancer cells expressing normal *PI3K* and *PTEN* genes, other lesions are present that activate the PI3K signalling network (that is, activated tyrosine kinases, RAS and AKT, as well as loss of liver kinase B1 (LKB1; also known as STK11), type II inositol polyphosphate-4-phosphatase (INPP4B) and tuberous sclerosis (TSC))[11]. This strong genetic evidence, in addition to the druggability of various components in the network, provided the original rationale and enthusiasm for targeting PI3K–AKT–mTOR signalling in oncology. The targeting of this signalling network was seen as an opportunity to combat tumour complexity and genomic heterogeneity through a central, common oncogenic driver that is fundamental to all cancer cells. However, counterbalancing this opportunity is the challenge of targeting enzymes that are also active and have crucial roles in normal cells and tissues.

Groundbreaking structural studies of PI3K enzymes[12–18], together with extensive medicinal chemistry efforts[19–21], have led to the discovery of compounds targeting one or more nodes in the network. Several of these compounds harbour favourable drug properties and suppress tumour growth in preclinical models of cancer[20–24]. The challenge is to translate these findings into a meaningful activity with acceptable tolerability in patients with cancer. The early results from clinical trials in advanced solid tumours are rather sobering, showing limited single-agent activity of PI3K and mTOR inhibitors[25,26], especially when compared to agents targeting driver oncogenes such as *BCR–ABL*, anaplastic lymphoma kinase (*ALK*) or *BRAF*. Pharmacology plays an important part in clinical efficacy, in that doses that are high enough and administered over a sufficiently long exposure period to

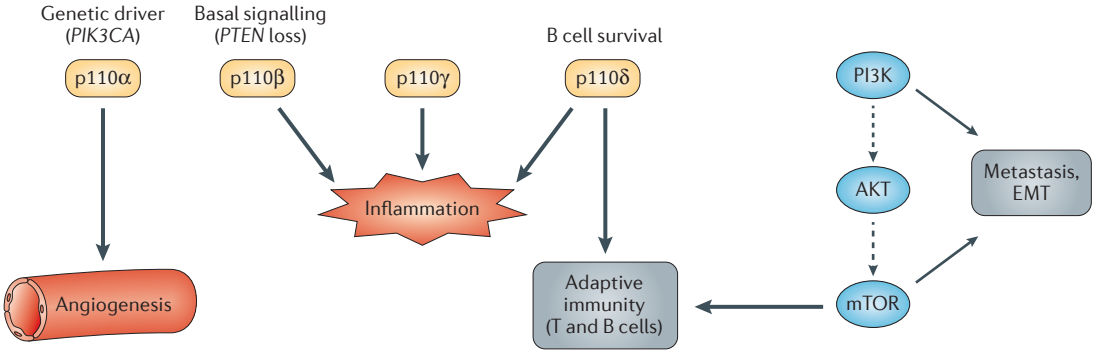


Figure 1 | **Targets in the signalling network and their role in tumour biology.** This diagram shows a highly simplified scheme of the signalling pathway leading from phosphoinositide 3-kinase (PI3K), to AKT, to mammalian target of rapamycin (mTOR). The four isoforms of class I PI3K are shown in orange boxes. The cancer-cell-intrinsic functions of the isoforms are illustrated above: the PI3K catalytic isoform p110α (encoded by *PIK3CA*) is a frequent genetic driver (*PIK3CA* mutations); basal activity of p110β is implicated in tumours with loss of phosphatase and tensin homolog (*PTEN*); and p110δ has a fundamental role in the survival of normal B cells and is implicated in malignancies of this lineage. PI3K and mTOR drive tumour metastasis by promoting cell motility and epithelial–mesenchymal transition (EMT). The bold arrows represent cell-extrinsic functions of various components in the network. p110α drives angiogenesis; p110γ, p110δ and p110β have important functions in inflammatory cells; and p110δ and mTOR control key aspects of adaptive immunity, including lymphocyte activation, differentiation and tolerance. Drugs in clinical development that target the nodes in this network are listed in Supplementary information S1 (table).

achieve cancer eradication might not be tolerated owing to mechanism-based on-target toxicities. Yet the pathway itself might not be as essential to cancer cells as originally proposed, at least at an advanced stage of tumorigenesis. Indeed, blockade of the pathway generally fails to induce cancer cell death and leads to selection for compensatory pathways that maintain survival and restore tumour growth[26–29]. Furthermore, refinement of genetically engineered mouse models suggests that *PIK3CA* mutants expressed at endogenous levels do not strongly drive tumour development in the same way as some other oncogenes[30,31]. In essence, oncogene addiction to PI3K–AKT–mTOR signalling is not absolute. Therefore, unleashing the full potential of PI3K–AKT–mTOR inhibitors in oncology will require earlier treatment, dose and schedule optimization as well as rational combinations with other therapeutic approaches.

It will also be important to identify biomarkers that can guide patient selection and to determine which tumour types or genetic profiles benefit from the blockade of single nodes and isoforms compared to multiple targets. Encouragingly, the p110δ-selective inhibitor GS-1101 (formerly known as CAL-101 and currently in Phase III development) produces dramatic responses in some B cell malignancies[32,33]. This proves the principle that a potent and selective PI3K inhibitor can improve the survival of selected patient populations in cancer. However, GS-1101 has an unusual mechanism of action: the drug is not directly cytotoxic to malignant B lymphoma cells and its efficacy arises in part from modulating the immune environment of the tumour[32–34]. This illustrates the importance of understanding the biology of the PI3K pathway in immune cells and in physiological models of tumour immunity (or immunology). The success of antibody therapies targeting immune checkpoints (such as cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death protein 1 (PD1))[35,36] emphasizes the potential of targeting immune-inhibitory pathways in cancer and the importance of evaluating the immune effects of small-molecule kinase inhibitors.

The goal of this Review is to reset both expectations and directions. Our understanding of the complexity of the PI3K–AKT–mTOR signalling network and its role in cancer has substantially increased, establishing the pathway as a challenging yet viable target in oncology. Much can be learned from clinical failures and the limited successes so far to chart a course for next-generation strategies. In our opinion, the enthusiasm and commitment towards targeting such an important pathway in cancer should not be dampened.

The PI3K–AKT–mTOR signalling network

Key features of the PI3K–AKT–mTOR signalling network that illustrate both the promise of this pathway and the challenges for targeting it in cancer have been previously discussed[11,21,37,38]. Below, we provide a brief overview of the functions and signalling mechanisms of members of the PI3K family of enzymes, highlighting their roles in cancer and issues faced in therapeutically targeting them.

There are eight mammalian PI3K enzymes, which are grouped into three classes[38]. The most important in cancer are the four class I enzymes, termed PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ. These are heterodimers of a 110 kDa catalytic subunit (p110α, p110β, p110γ or p110δ) and a regulatory subunit. The catalytic isoforms share considerable sequence homology and produce the same lipid product (PtdIns(3,4,5)P_3), and each can receive activation inputs from both tyrosine kinases and GTPase signalling[38,39]. However, the details of these inputs differ (BOX 1).

PIK3CA
The human gene encoding the p110a catalytic isoform of phosphoinositide 3-kinase (PI3K). Gain-of-function mutations in *PIK3CA* are frequent in cancer.

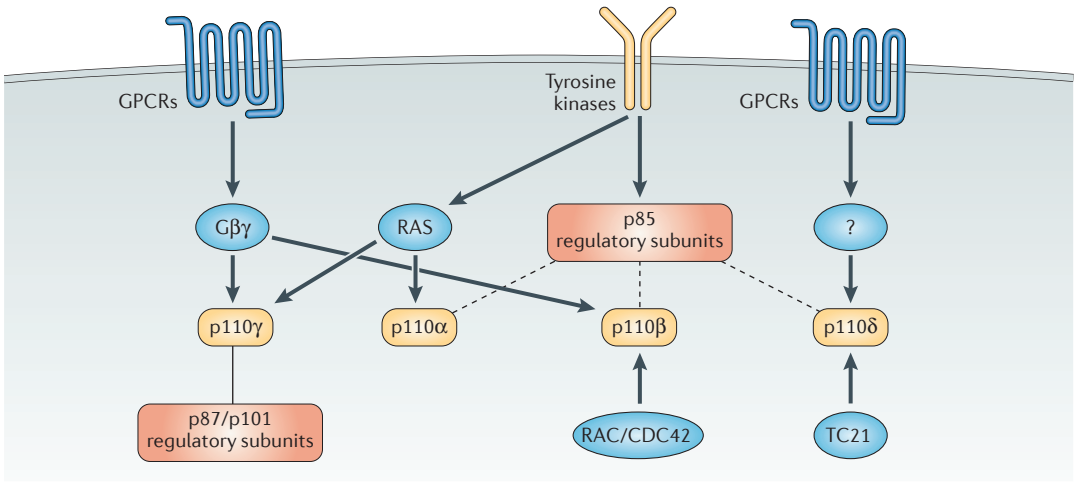
Phosphatase and tensin homolog
(PTEN). A lipid 3-phosphatase that opposes phosphoinositide 3-kinase (PI3K) signalling and is often disabled in cancer.

RAS
A family of small GTPases that are often mutated in cancer and activate phosphoinositide 3-kinase (PI3K) and other oncogenic signals.

Box 1 | **Inputs from GTPases and tyrosine kinases to PI3K**

Each of the class I phosphoinositide 3-kinase (PI3K) catalytic isoforms has a segment known as the RAS-binding domain (RBD). Knock-in mouse studies established the PI3K catalytic isoform p110α as a bona fide downstream effector of oncogenic RAS[76] and demonstrated that the RBD of p110γ is required for PI3K signalling in neutrophils[211]. Recent work indicates that the RBD of p110β does not bind to RAS or its closely related isoforms; instead, the RBD of p110β interacts with GTP-bound RAC and cell division cycle 42 (CDC42), establishing p110β as a novel effector of these GTPases[39].

The RBD of p110δ interacts with the small G protein TC21 (also known as RRAS2)[212,213]. Tyrosine kinases activate p110α, p110β and p110δ through the interaction of their regulatory subunits with tyrosine-phosphorylated peptide motifs, and can activate p110γ in some cell types via RAS[92]. G protein-coupled receptors (GPCRs) directly stimulate p110β and p110γ via βγ subunits of heterotrimeric G proteins[211,214], and can activate p110δ in B cells by an unknown mechanism[215,216].



The distinct activation mechanisms of the class I PI3K isoforms suggest that each isoform has unique biological functions — a model supported by abundant evidence from targeted gene inactivation in mice[38,40–43]. It follows that targeting single isoforms might have therapeutic effects. Conversely, functional redundancy in maintaining cell survival has been documented in various cell types, including cancer cells[44]. Furthermore, genetic mouse models have caveats and do not always accurately predict the response to acute target inhibition by pharmacological agents.

Of the four class I catalytic isoforms, only *PIK3CA* (encoding p110α) is frequently mutated in human cancer[8,11]. Although many *PIK3CA* mutations exist, there are two hotspots that cause elevated PI3K enzyme activity through distinct mechanisms[15]. Transforming mutations in the ubiquitously expressed gene *PIK3CB* (encoding p110β) are rare[45], perhaps because of this isoform's distinct mode of interaction with regulatory subunits[18]. Mutations in class I regulatory subunit genes (*PIK3R1* or *PIK3R2*) are also found in cancer cells and cause increased PI3K activity[46,47]. In cell transformation assays, p110α has a dominant role in the oncogenic potential of *PIK3R1* mutants[48]. This observation provides further support for a unique role of the p110α isoform in tumorigenesis. In addition, p110α has a cell-extrinsic role in tumour angiogenesis (FIG. 1) and possibly stromal fibroblasts[3,4], which demonstrates another potential advantage of targeting this isoform. In cancer cells with wild-type *PI3K* genes, there are usually oncogenic lesions in upstream tyrosine kinases and/or RAS that cause constitutive signalling through PI3K[11]. Loss of the lipid phosphatases *PTEN* and/or *INPP4B* is an alternative path to the elevation of PI3K lipid products, but the inactivation of these tumour suppressors is not mutually exclusive with mutations in PI3K or RAS[46,49]. Indeed, a mouse model demonstrated that loss of *PTEN* enhances the potential of *PIK3CA* mutations to cause ovarian tumours[30]. *PIK3CA* mutations or *PTEN* loss can also coexist with oncogenic tyrosine kinases[50,51].

The serine/threonine kinase mTOR functions at two distinct nodes in the PI3K signalling network[52,53] (FIG. 2a). mTOR complex 2 (mTORC2) phosphorylates key residues to activate AKT and other kinases. mTORC2 seems to have basal activity that is stimulated by growth factors and through its association with ribosomes[54]. mTORC1 is a central regulator of cellular metabolism and biosynthesis, and is subject to complex regulation by growth factors, nutrients and cellular stresses[53]. When conditions are favourable for cell growth, mTORC1 phosphorylates several substrates to promote anabolic processes (such as ribosome biogenesis, translation and the synthesis of lipids and nucleotides) and suppress catabolic processes (such as autophagy)[53]. One of the key control nodes for mTORC1 activity is the TSC complex containing TSC1, TSC2 and TBC1 domain family member 7 (TBC1D7) proteins[55,56]. By phosphorylating TSC2, AKT suppresses the inhibitory effect of the TSC complex on mTORC1. Although the *MTOR* gene is not frequently mutated in human tumours, there is evidence for

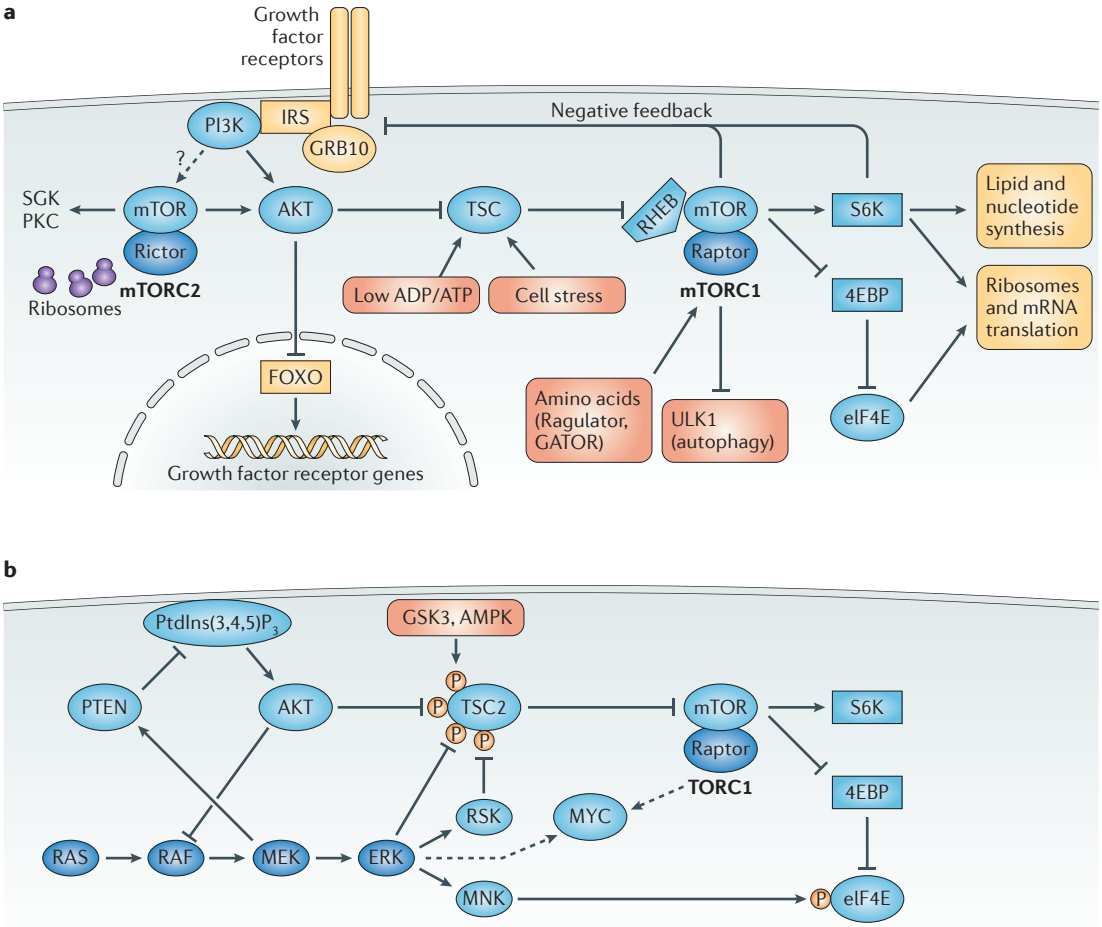


Figure 2 | **Complexity, crosstalk and feedback in the PI3K–AKT–mTOR signalling network. a** | The mammalian target of rapamycin complex 1 (mTORC1)–mTORC2 network and key feedback mechanisms. The serine/threonine kinase mTOR forms two multiprotein complexes whose defining subunits are Raptor (regulatory-associated protein of mTOR) and Rictor (rapamycin-insensitive companion of mTOR). mTORC2 activity is stimulated by association with ribosomes and by growth factors through a poorly defined mechanism, which may involve phosphoinositide 3-kinase (PI3K). mTORC2 promotes the stability and activity of AKT and other kinases, including serum- and glucocorticoid-induced kinases (SGKs) and protein kinase C (PKC). mTORC1 is a signal integrator whose activity is fine-tuned by diverse inputs. Growth factors, energy sensors and cellular stress converge at the level of the tuberous sclerosis (TSC) complex (which includes TSC1, TSC2 and TBC1 domain family member 7 (TBC1D7)), which is a negative regulator of mTORC1 that has GTPase-activating protein (GAP) activity towards the RHEB GTPase. Amino acids regulate mTORC1 through the Ragulator and GATOR (GAP activity toward RAGs) complexes. mTORC1 promotes anabolic programmes through many substrates, of which three classes are shown: S6 kinases (S6Ks), eIF4E-binding proteins (4EBPs) and autophagy regulators (such as UNC51-like kinase 1 (ULK1), and so on). mTORC1 activity exerts feedback control on growth factor signalling. One canonical feedback pathway is initiated by S6K1, an mTORC1 substrate that phosphorylates adaptor proteins of the insulin receptor substrate (IRS) family to attenuate growth factor receptor signalling to PI3K and RAS. In parallel, mTORC1 suppresses growth factor receptor signalling by phosphorylating the adaptor protein growth factor receptor-bound protein 10 (GRB10). AKT activity triggers a feedback mechanism that suppresses growth factor receptor expression and signalling. Through phosphorylation and inactivation of forkhead box O (FOXO) transcription factors, active AKT reduces the transcription of FOXO target genes, including several growth factor receptors. **b** | Redundancy and feedback between the RAS–RAF–MEK (MAPK/ERK kinase)–ERK (extracellular signal-regulated kinase) and PI3K–AKT–mTOR signalling networks. ERK and the downstream kinase RSK (ribosomal S6 protein kinase) can compensate for AKT in the activation of mTORC1 via inhibitory TSC2 phosphorylation; glycogen synthase kinase (GSK3)- and AMP-activated protein kinase (AMPK)-induced phosphorylation of TSC2 increase its ability to suppress mTORC1 activity. MNK kinases phosphorylate eukaryotic translation initiation factor 4E (eIF4E) to provide a distinct signal to increase cap-dependent translation. ERK and mTOR independently promote the accumulation of MYC oncoproteins. Mutual feedback inhibition is a feature of the two pathways: MEK activity suppresses PI3K signalling by promoting the membrane localization of phosphatase and tensin homolog (PTEN), whereas AKT activity suppresses RAS activation through the mechanisms shown in FIG. 3. AKT can also phosphorylate and inhibit RAF[227,228]. PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate (also known as PIP_3).

non-oncogene addiction to mTOR function in cancer cells. For example, tissue-specific deletion of mTOR in the mouse prostate inhibits tumour formation driven by PTEN loss without disrupting normal prostate tissue[57,58]. Also, mTOR catalytic inhibitors can achieve antileukaemic effects at doses that preserve the function of normal bone marrow and peripheral lymphocytes[59,60].

Feedback control is a common feature of cellular signalling systems, and the PI3K–mTOR network provides many examples of this (FIG. 2a). An important consequence of feedback control is that inhibitors of AKT or mTOR tend to cause elevated expression and activity of growth factor receptors, leading to increased PI3K activity and RAS signalling, and activation of alternative survival pathways in cancer cells[61,62]. There are several potential strategies to overcome the 'rebound' signalling in response to PI3K–AKT–mTOR inhibitors, including inhibition of several signalling nodes and combination approaches.

There is also crosstalk between elements of the PI3K signalling network and components of other oncogenic pathways (FIG. 2b). A key consequence is that PI3K and AKT are not the dominant regulators of TSC1, TSC2 and mTORC1 in some cells. Extracellular signal-regulated kinase (ERK) and ribosomal protein S6 kinase (RSK) are two effector kinases downstream of RAS that can promote mTORC1 activity by phosphorylating TSC2 on residues that are distinct from AKT phospho-acceptor sites[63–65]. Glycogen synthase kinase 3 (GSK3) and AMP-activated protein kinase (AMPK) can also phosphorylate TSC2 (REF. 66). Another example of crosstalk is that ERK and mTORC1 provide distinct and complementary inputs to eukaryotic translation initiation factor 4E (eIF4E), which is a central regulator of cap-dependent mRNA translation[67,68]. The PI3K–AKT–mTOR and RAS–RAF–MEK (MAPK/ERK kinase)–ERK networks also converge to stabilize the expression of the MYC oncoprotein[69]. Therefore, oncogenic compensation by RAS can severely limit the anticancer efficacy of PI3K–AKT–mTOR inhibitors. Conversely, active PI3K signalling is probably a central mechanism of resistance to various targeted therapies.

Clinical trial results and associated challenges

There are six general classes of agents in clinical trials targeting the PI3K–AKT–mTOR network: pan-class I PI3K inhibitors, isoform-selective PI3K inhibitors, rapamycin analogues (rapalogues), active-site mTOR inhibitors, pan-PI3K-mTOR inhibitors and AKT inhibitors. Supplementary information S1 (table) lists many of the compounds that are currently in oncology clinical trials according to the ClinicalTrials.gov database. Rapalogues are not broadly effective as single agents, although they have been approved by the US Food and Drug Administration (FDA) for the treatment of a few tumour types for which modest therapeutic effects can be achieved. Clinical trial data for the other five classes remain largely unpublished; however, some preliminary conclusions can be made on the basis of results presented at conferences.

The most impressive results have been achieved with the p110δ-selective inhibitor GS-1101 (idelalisib), which causes dramatic responses in chronic lymphocytic leukaemia (CLL) and certain other B cell malignancies (BOX 2). Overall, other agents targeting the PI3K–AKT–mTOR pathway have not yielded broad responses when given, at tolerated doses, to patients with advanced-stage cancer. By comparison, early trials of currently approved drugs targeting oncogenes such as *BCR–ABL*, mutant *BRAF* or *ALK* revealed marked single-agent activity even in Phase I trials, albeit in prospectively selected patient populations. A recent review by Tabernero and colleagues[25] provided a detailed discussion of emerging clinical trial data for PI3K pathway inhibitors, including safety profiles and pharmacodynamic markers. Below, we highlight four central challenges and issues in the field of PI3K–AKT–mTOR drug development arising from clinical studies carried out so far.

Pan-PI3K versus isoform-selective inhibition. Several pan-class I PI3K inhibitors in clinical trials target all four class I PI3K isoforms with similar potencies (Supplementary information S1 (table)). The main argument in support of pan-PI3K inhibitors is that most cancer cells express multiple PI3K isoforms with redundant functions in oncogenic signalling[44]. Another factor driving the early development of pan-PI3K compounds was that these efforts proceeded before PI3K isoform structures were obtained to aid the design of isoform-selective compounds. However, pan-PI3K inhibitors are blunt tools that are not specifically aligned with the disease biology and context. The main concern associated with pan-PI3K inhibitors is that the doses needed to fully block all class I PI3Ks for extended periods might not be tolerated. For this reason, it is possible that clinical trials to date have missed an 'all or nothing' threshold for tumour responses owing to dose-limiting toxicities. A related concern is that the first-in-class compounds that have entered oncology trials are not sufficiently selective for PI3K. Compared to isoform-selective inhibitors, compounds targeting all class I PI3Ks more commonly seem to have off-target effects on other members of the PI3K-related kinase (PIKK) family (which includes mTOR, DNA-dependent protein kinase catalytic subunit (DNA-PK), ataxia telangiectasia mutated (ATM) as well as ataxia telangiectasia and RAD3 related (ATR)) and other cell components. For example, at the concentrations needed to fully inhibit PI3K, BKM120 has off-target effects on tubulin and causes general cellular toxicity[70]. Filling the competitive landscape with inadequate compounds might have discouraged the later entry of best-in-class agents with the required selectivity to deliver on the potential of the target biology.

Isoform-selective PI3K inhibitors (Supplementary information S1 (table)) have the potential to completely block the relevant target while limiting toxicities associated with broader inhibition profiles. Indeed, GS-1101 is well tolerated in most patients when administered at doses that maintain drug exposure at levels that are sufficient to suppress p110δ activity and that translate into antitumour activity[33]. Yet the therapeutic activity of p110δ inhibitors was unexpected. These compounds deviate from the traditional paradigm for targeting a kinase that is required for the cancer cell but not its

Rapalogues
Structural analogues of rapamycin that inhibit mammalian target of rapamycin (mTOR) but have altered pharmacological properties.

Pan-PI3K inhibitors
Molecules that inhibit all class I phosphoinositide 3-kinase (PI3K) enzymes but are selective relative to other lipid and protein kinases.

Box 2 | **Selective inhibitors of PI3Kδ or the PI3K effector BTK**

The phosphoinositide 3-kinase δ subunit (PI3Kδ) isoform is mainly expressed in immune cells and is absent from most solid tumours. Gene targeting in mice has established essential functions for PI3Kδ in mature B cells and in other immune cell types[43,217]. A key downstream effector of PI3Kδ in B cells is Bruton's tyrosine kinase (BTK), which is a member of the TEC family of non-receptor tyrosine kinases. PI3Kδ and BTK are activated by signals from the B cell receptor (BCR), chemokines and cytokines to drive survival, proliferation and adhesion to supportive stromal cells. However, activating mutations in PI3Kδ and BTK are not present in B cell tumours, and inhibitors of these enzymes were initially developed for application in immune diseases. Unexpectedly, Phase I clinical trials of a PI3Kδ inhibitor (CAL-101, renamed GS-1101) and a BTK inhibitor (PCI-32765) showed dramatic and durable responses in a subset of patients with indolent B cell malignancies[32,33,71,72]. Even greater efficacy was achieved in combination studies with rituximab and/or bendamustine. Both the PI3Kδ and BTK inhibitors have shown acceptable safety profiles. These compounds, now called idelalisib and ibrutinib, have progressed to Phase II/III trials and are likely to be the first US Food and Drug Administration (FDA)-approved agents targeting the PI3K pathway. The FDA has granted breakthrough therapy designation for ibrutinib in three diseases: mantle cell lymphoma, Waldenström's macroglobulinaemia and chronic lymphocytic leukaemia with deletion at chromosome 17p. Ibrutinib was approved on 13 November 2013 for the treatment of relapsed mantle cell lymphoma.

normal counterpart. In this case, the target (*PIK3CD*) is not mutated in cancer but is required for the survival of normal B cells (FIG. 1). The efficacy of GS-1101 derives from an unusual confluence of factors: a very selective drug with a target that has a restricted expression, and a dual role for the target in the cancer cell and the tumour immune environment. This emerging paradigm for leukaemia and lymphoma treatment also applies to ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK) that acts downstream of p110δ in B cells[71,72].

Other than p110δ inhibitors, the most advanced isoform-specific compounds are selective for p110α (Supplementary information S1 (table)). The prevalence of *PIK3CA* mutations in human cancer provides a potentially rapid and cost-effective development path analogous to that of BRAF or ALK inhibitors. Yet, questions remain about the best patient selection strategy for p110α-selective inhibitors. One approach is to design basket trials grouping patients with *PIK3CA*-mutant tumours across several histologies, and allow the data to guide expanded trials. This idea builds on experience attained from the use of BRAF inhibitors, which provided efficacy in BRAF-mutant melanoma but not in colorectal cancer[73,74]. Similarly, the efficacy of GS-1101 in CLL emerged from empirical testing in a broad range of B cell malignancies[32,33]. Another approach is to include tumour types that express wild-type *PIK3CA* but in which p110α has a crucial signalling role (for example, HER2 (also known as ERBB2 and Neu), KRAS and PI3K regulatory subunit 1 (PIK3R1))[48,75,76]. With either approach, drugs targeting p110α should be tested in patients at an earlier stage of disease with less tumour complexity and a reduced toxicity load from prior treatments.

There is also an opportunity, so far untapped, to develop irreversible p110α inhibitors, as these are the only isoforms with a reactive cysteine residue near the ATP binding site[77]. The clinical success of the covalent BTK inhibitor ibrutinib supports this pharmacological approach[71]. Another way to improve the therapeutic index might involve developing inhibitors that are selective for the common *PIK3CA* 'hotspot' mutant enzymes such as H1047R, E542K and E545K. However, such compounds would lose cell-extrinsic activity (for example, angiogenesis), they would not act on wild-type p110α downstream of receptors and RAS, and they might select for other mutants. Agents targeting hotspot *PIK3CA* mutants might find an alternative use in the treatment of inherited overgrowth syndromes caused by somatic *PIK3CA* mutations[78–80]. Another clinical use of isoform-selective agents outside of oncology might be the use of p110δ inhibitors in patients with newly identified immunodeficiency syndromes caused by activating mutations in *PIK3CD*[81,82].

Some studies suggest that p110β activity is essential in cancer cells lacking PTEN (FIG. 1), particularly in prostate and breast cancer[83–85], which suggests that p110β inhibitors would be more effective than p110α inhibitors in patients with PTEN-deficient tumours. However, another study reported that p110α and p110β have overlapping functions in various PTEN-deficient tumour models[86]. p110α also has the aforementioned role (FIG. 1) in tumour angiogenesis[3,4]. Ultimately, the success of targeting p110β alone in PTEN-mutant advanced tumours may depend on whether the tumour also harbours mutations in upstream receptors or RAS that activate p110α.

Arguments can be made for compounds targeting two of the four class I isoforms, and this now seems technically feasible as a result of advances at the level of structural biology and medicinal chemistry. A dual p110α–p110β inhibitor might be effective in tumours lacking PTEN or in *PIK3CA*-mutant tumours that have become resistant to single p110α inhibition. However, targeting both p110α and p110β is likely to recapitulate most of the toxicity profile seen with pan-PI3K inhibitors. A compound targeting p110α and p110δ might overcome resistance to GS-1101 in B cell malignancies, as in some cases resistance correlates with elevated p110α expression and activity[87]. A dual p110α–p110δ inhibitor, BAY 80–6946, was recently described[88]. Combined targeting of p110γ and p110δ has potential in T cell leukaemias, in which p110γ and p110δ have redundant functions[89]. The compound IPI-145 is selective for p110γ and p110δ (but it is tenfold more potent towards p110δ), has activity in autoimmunity models[90,91] and is in clinical trials for both B and T cell malignancies.

Basket trials
Clinical trials that enrol patients with tumours of diverse tissue and histological origin, but with a shared genetic signature (in this case, PI3K catalytic isoform p110α (*PIK3CA*)-mutant tumours).

Most solid tumour cells express p110α and/or p110β, but usually not p110γ or p110δ. Nevertheless, inhibiting p110γ or p110δ may suppress tumour viability by modulating leukocyte subsets in the tumour environment (FIG. 1). In mouse models, blocking p110γ activity reduces the recruitment of inflammatory cells to tumour sites and suppresses tumour growth[92]. Whether p110γ inhibition can shrink established tumours is uncertain, but this approach could be used to prevent regrowth or metastasis. Inhibiting p110δ suppresses the function of regulatory T cells, enabling increased cytotoxic T cell responses to tumours (K. Ali, K. Okkenhaug and B. Vanhaesebroeck, personal communication). Thus, targeting p110α with p110δ in solid tumours seems to be a particularly promising approach that would have cell-intrinsic anticancer effects, while promoting a favourable immune environment and avoiding some of the toxicities associated with pan-PI3K inhibition. A downside of pan-PI3K inhibitors is that they suppress the function of mouse and human lymphocytes to a greater degree than p110α inhibition alone or p110α–p110δ combinations[93]. It is likely that compounds with seemingly subtle differences in potency against different isoforms will provide substantially different efficacy and tolerability based on cancer cell-extrinsic effects.

Single-node versus pan-PI3K and mTOR inhibition. mTOR is structurally related to PI3Ks, and many ATP-competitive compounds inhibit mTOR and PI3K with similar potencies. In fact, the broadly used experimental PI3K inhibitors wortmannin and LY294002 also directly inhibit mTOR[94–96]. Several pan-PI3K–mTOR inhibitors with improved pharmacological properties are now in clinical trials (Supplementary information S1 (table)). The rationale for this compound class is to overcome cross-talk and feedback through inhibition of the pathway at three key nodes: PI3K, mTORC1 and mTORC2 (FIG. 2a). This approach circumvents a limitation of selective PI3K inhibitors: that is, other inputs maintain considerable mTORC1 activity even when PI3K and AKT are switched off[97]. Pan-PI3K and mTOR inhibitors should also prevent the rebound activation of PI3K that occurs in cells treated with rapalogues or active-site mTOR inhibitors.

It seems unlikely that pan-PI3K and mTOR inhibitors will provide a better efficacy window than agents targeting single nodes, as there is obviously potential for higher toxicity at effective doses. Another consideration is that pan-PI3K and mTOR inhibitors do not always provide better efficacy than selective active-site mTOR inhibitors in preclinical tumour models[60]. Combined targeting of mTOR and one PI3K isoform (for example, p110α in *PIK3CA*-mutant tumours) might improve tolerability relative to pan-PI3K and mTOR inhibitors and increase efficacy compared to single PI3K inhibition. A combination of the RAF and MEK inhibitors dabrafenib and trametinib has greater efficacy in metastatic melanoma than monotherapy, which provides proof of concept in a different oncogenic pathway[98]. In addition to suppressing intra-pathway feedback, such combination approaches may achieve synergistic suppression of key downstream effectors using synchronized doses that partially inhibit the two upstream targets. A Phase Ib trial (ClinicalTrials.gov identifier: NCT01899053) has been initiated by Millennium Pharmaceuticals to test a combination of inhibitors that are selective for p110α and mTOR (MLN1117 and MLN0128). Providing further support for such combinations, the resistance of *PIK3CA*-mutant breast cancers to BYL719 correlated with persistent mTORC1 signalling[99]. In the dabrafenib/trametinib trial, phosphorylated ERK was a reliable downstream pharmacodynamic marker and we anticipate that pS6 and phosphorylated eIF4E-binding protein 1 (4EBP1) offer similar potential as pharmacodynamic markers for mTORC1 activity.

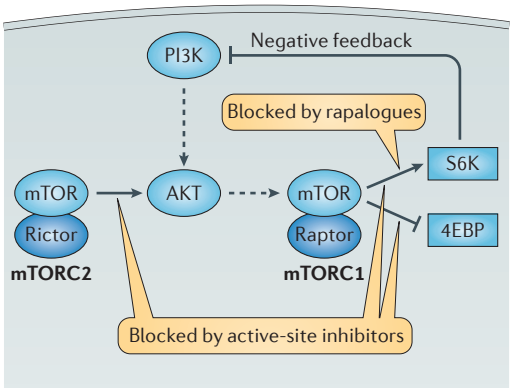
Rapalogues versus mTOR kinase inhibitors. Rapalogues (everolimus, temsirolimus and deforolimus) are structural analogues of rapamycin with improved pharmacological properties[100]. The acceptable safety profile[101] of rapalogues has allowed the completion of several clinical trials testing these compounds as single agents or in combination. Although most single-agent trials have not demonstrated therapeutic benefit, rapalogue monotherapy does considerably extend survival for some patients with cancer. Currently, one or more rapalogues are approved by the FDA for use in renal cell carcinoma, mantle cell lymphoma and neuroendocrine tumours.

Incomplete mTOR inhibition contributes to the limited efficacy of single-agent rapalogues in cancer (BOX 3). A second strategy to target mTOR is through ATP-competitive inhibitors that completely block mTOR kinase activity in both complexes — mTORC1 and mTORC2 (REFS 20,102,103) (BOX 3). Termed active-site mTOR inhibitors, these compounds cause greater suppression of biosynthetic pathways than rapamycin, and generally cause a more marked cytostatic effect in cell lines[20,102–104]. Active-site mTOR inhibitors have shown cytotoxic effects in some but not all preclinical cancer models[60,105,106]. A key question for clinical development is whether active-site mTOR inhibitors should be tested first in malignancies in which rapalogues have some clinical benefit, as mTOR is a validated target in those diseases. An alternative approach is to test more broadly to see whether complete mTORC1 and mTORC2 inhibition is effective in diseases in which partial mTORC1 inhibition is not. It is important to note that most of the encouraging preclinical results with active-site mTOR inhibitors have been achieved in combination with tyrosine kinase inhibitors in mouse xenograft models of human tumours[60,107]. These findings argue for the early evaluation of active-site mTOR inhibitors in combination with other targeted agents. A note of caution is that advanced tumours often have an increased ratio of eIF4E to 4EBPs, which is a known mechanism of resistance to active-site mTOR inhibitors[108,109]. Therefore, high eIF4E expression might be a useful negative prognostic biomarker for patient selection.

Recent findings have renewed interest in the clinical application of rapalogues. A large combination trial (BOLERO-2) of everolimus with anti-oestrogen therapy (aromatase inhibitors) showed a statistically significant survival benefit for patients with hormone receptor-positive breast cancer[110]. Rapalogues are useful in the

Box 3 | **Two distinct classes of mTOR inhibitors**

Rapalogues act through an allosteric mechanism and cause only partial inhibition of mammalian target of rapamycin complex 1 (mTORC1); they have more marked effects on certain mTORC1 substrates (that is, S6 kinase 1 (S6K1)) than others (that is, eIF4E-binding proteins (4EBPs)). This profile causes weak inhibition of cap-dependent translation and releases negative feedback, leading to 'rebound' activation of upstream signalling. Rapalogues do not directly inhibit mTORC2, allowing continual survival signalling by AKT and other mTORC2 substrates. In accord, extensive cell line surveys consistently show that rapamycin and its analogues are cytostatic and not cytotoxic. Active-site mTOR inhibitors fully block the phosphorylation of all known mTORC1 and mTORC2 substrates.



PI3K, phosphoinositide 3-kinase.

treatment of subependymal giant cell tumours and angiomyolipomas in patients with tuberous sclerosis caused by inherited mutations in the *TSC1* or *TSC2* genes[111,112]. Another important study used deep genomic sequencing of a rare responder tumour to identify *TSC1* loss as a biomarker of sensitivity to everolimus in bladder cancer[113]. Novel tumour suppressors such as nitrogen permease regulator 2-like protein (NPRL2) and DEP domain-containing protein 5 (DEPDC5) have been identified in the GATOR1 (GAP activity towards RAGs) complex that regulates amino acid sensing by mTORC1, and human cancer cell lines lacking these components are very sensitive to rapamycin[114,115]. Additional clinical studies are needed to determine whether loss of TSC or GATOR1 components predicts sensitivity to rapalogues in a broad range of tumours. Finally, two recent studies identified mTORC1 signalling in resistance to PI3K or BRAF inhibitors[99,116].

The strong immunosuppressive properties of rapamycin have led to extensive investigation of mTOR function in the immune system. Genetic and pharmacological studies in mice have shown remarkable complexity of mTOR function in different immune cell types and at different stages of cell activation[117–119]. Although mTOR blockade reduces the proliferation and effector differentiation of CD4 T cells, mTOR inhibition enhances the generation of CD8 memory T cells[120–122]. In addition, mTOR inhibition can augment inflammatory cytokine production by innate immune cells[123]. Remarkably, mTOR inhibition can either promote or suppress the function of regulatory T cells, depending on the timing and experimental conditions[122,124,125]. Therefore, modulating the schedule of mTOR inhibitor therapy has the potential to promote antitumour immune responses while providing some tumour-intrinsic activity.

Tolerability and alternative targets. A recurring theme, as discussed above, is the challenge of achieving a therapeutic window for compounds targeting PI3K and/or mTOR. PI3K signalling is linked to many physiological processes, and mTOR is a non-redundant sensor of nutrients and growth factors in dividing cells[53,126,127]. For these reasons, many investigators have evaluated other targets in the PI3K–mTOR signalling network. The best studied of these is AKT. This kinase is commonly overexpressed or mutated in tumours and was first discovered as the oncogene of a transforming virus[128]. As AKT is one of many PI3K effectors linked to cell-specific physiological functions, it is conceivable that direct AKT inhibition would attack cancer cells with greater selectivity than PI3K inhibition. However, the data so far suggest that this might not be the case. AKT inhibitors cause severe rash, like some tyrosine kinase inhibitors, and they cause hyperglycaemia in both mice and humans[129,130]. Genetic studies in mice have shown that the AKT2 isoform is required for insulin signalling[131], and most clinical AKT inhibitors block both the AKT1 and AKT2 isoforms[132]. Conversely, some AKT inhibitor candidates have off-target effects, and a recent study suggested that a pharmacologically optimized AKT inhibitor causes only transient and reversible hyperglycaemia[133]. Hyperglycaemia can be managed with approved drugs such as metformin, and it can also be a useful biomarker of target modulation. Interestingly, it has been suggested that metformin and related compounds provide direct antitumour effects by activating AMPK, which leads to reduced mTORC1 signalling[134].

Several other cellular components associated with the PI3K–mTOR network might be useful targets for anticancer therapeutics (TABLE 1). Based on the central role of cap-dependent translation in cancer cells, drugs targeting eIF4E have been developed and show promise in preclinical studies[135]. For example, the compound 4EGI-I interferes with the eIF4E–eIF4G interaction and has anticancer activity in cell lines[136]. Selective inhibitors of S6 kinases (S6Ks) have been identified[137–139] and could attack cancer by restricting protein synthesis and other anabolic growth processes mediated by S6Ks. Supporting this concept, genetic targeting of S6K1 delayed leukaemogenesis in a PTEN-deficient model[140]. MNK and PIM kinases also promote protein synthesis and are under evaluation as targets in oncology[141,142]. MAPK-interacting kinases (MNKs) phosphorylate eIF4E to increase cap-dependent translation and promote survival[143], whereas PIM kinases increase translation by phosphorylating several substrates including eIF4B[142]. Inhibiting RAS function in RAS-driven cancers would

Table 1 | **Emerging targets within the PI3K signalling network**

Target	Upstream activators	Effectors or substrates	Tool compounds	Refs
eIF4E	mTORC1, MNK	Cap-dependent translation	4EGI-I	136
			4ei-1	218
S6K	mTORC1, PDK1	S6, PDCD4, eIF4B, eEF2K, POLDIP3	PF-4608671	139
			DG2	138
			LYS6K2	137
MNK	ERK	eIF4E	CGP57380	219
			AST 487	220
			Cercosporamide	221
PIM	Growth factor-mediated increase in transcription	eIF4B, 4EBP1, BAD, p27	SMI-4a	222
			ETP-45299	223
			SGI-1776	224
			Pimi-14J	225
			K00135	226

4EBP1, eIF4E-binding protein 1; BAD, BCL-2 antagonist of cell death; eEF2K, eukaryotic elongation factor 2 kinase; eIF4E, eukaryotic translation initiation factor 4E; ERK, extracellular signal-regulated kinase; mTORC1, mammalian target of rapamycin complex 1; PDCD4, programmed cell death protein 4; PDK1, 3-phosphoinositide-dependent protein kinase 1; PI3K, phosphoinositide 3-kinase; POLDIP3, polymerase delta-interacting protein 3 (SKAR); S6K, S6 kinase.

be expected to diminish signalling through PI3K as well as ERK and other RAS effectors. Although targeting RAS has long been an unrealized dream in molecular medicine, novel strategies have recently been described[144,145].

Emerging rational combination strategies

The typical drug development path for a targeted anticancer drug involves establishing single-agent efficacy before testing the drug in combination. However, initial results from trials of PI3K–AKT–mTOR inhibitors suggest that deep and sustained responses to single agents are infrequent. Given the limited resources, the development of some promising drugs might be halted because they do not significantly prolong the survival of a selected patient population. An alternative approach would be to initiate combination trials as soon as pharmacodynamic activity can be established at a tolerated dose. The development of robust and informative pharmacodynamic markers remains a challenge, as discussed in REF. 25. Moreover, it is essential to choose rational combinations that are most likely to provide synergy (a '1 + 1 = 3' effect), to overcome the expected increases in toxicity and justify the costs and complexity of combination trials. The BOLERO-2 clinical trial combining everolimus with endocrine therapy provided proof of principle that the PI3K–AKT–mTOR pathway can be targeted in rational combinations to achieve real therapeutic benefit to a large patient population[110]. What other combinations (TABLE 2) can be envisioned?

There is ample mechanistic rationale to test combinations of PI3K–AKT–mTOR inhibitors with tyrosine kinase inhibitors. It is useful to consider this issue from two perspectives (FIG. 3). First, cancers harbouring active or overexpressed receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR) or HER2 can display resistance to tyrosine kinase inhibitors through PI3K signalling[146,147]. This knowledge provides justification for adding PI3K–AKT–mTOR inhibitors to initial tyrosine kinase inhibitor treatments to prevent the emergence of resistance, even in tumours with a high initial response rate to tyrosine kinase inhibitors. In line with this view, a review[148] recently discussed strategies for incorporating PI3K inhibitors into treatment regimens for HER2-positive breast cancer. Such approaches should be considered for other tyrosine kinase-driven cancers; for example, combining GDC-0941 with imatinib produced more durable remissions than imatinib alone in a xenograft model of a gastrointestinal stromal tumour (GIST) driven by BCR–ABL[149].

A second consideration is that single-agent PI3K–AKT–mTOR inhibitors increase RTK expression through forkhead box O (FOXO)-mediated feedback[28,62]. From this point of view, tyrosine kinase inhibitors act as the second agent to augment the efficacy of a PI3K–AKT inhibitor. Supporting this concept, targeting members of the EGFR family with lapatinib increased the efficacy of a p110α-selective inhibitor in *PIK3CA*-mutant breast cancer cells[150]. A challenge is that the feedback tends to increase the expression of multiple RTKs, such that selective tyrosine kinase inhibitors would have minimal efficacy. In haematopoietic malignancies driven by non-RTKs such as BCR–ABL or Janus kinase 2 (JAK2), PI3K–AKT–mTOR inhibitors strongly synergize with tyrosine kinase inhibitors[60,151]. A possible explanation for this synergy is that blood cancer survival is maintained in part by cytokines and stromal cell contacts that signal through the PI3K pathway. There is also evidence for the interdependence of PI3K signalling and the JAK–STAT (signal transducer and activator of transcription) pathway; agents that target STAT3 or upstream kinases hold promise for enhancing the efficacy of PI3K inhibitors[152].

Targeting the RAS–RAF–MEK–ERK cascade is an attractive strategy for combination therapies with PI3K–AKT–mTOR inhibitors (FIG. 2b). Both networks can promote cell proliferation and survival, and there is extensive crosstalk between the pathways. Thus, mTORC1 inhibition tends to increase ERK phosphorylation[153,154], whereas MEK inhibition reduces PTEN membrane localization and increases AKT activity[155]. Synergy of a MEK inhibitor with the dual PI3K–mTOR inhibitor NVP-BEZ235 was first shown in a *KRAS*-driven lung cancer model[27]. Similar findings have been observed in many subsequent reports, including a study of *NRAS*-mutant melanoma cells[156]. One mechanism for synergistic cell killing appears to be through complementary effects on pro-apoptotic proteins: MEK–ERK inhibition stabilizes BCL-2-interacting mediator of cell death (BIM), whereas PI3K–AKT inhibition upregulates p53-upregulated modulator of apoptosis (PUMA; also known as BBC3) via FOXO transcription factors[157]. Both pathways also converge on the pro-apoptotic protein BCL-2 antagonist of cell death (BAD)[158]. Such combinations might also achieve synergy at the level of metastasis suppression. MEK inhibitors can suppress epithelial–mesenchymal transition (EMT), which is a crucial step

Table 2 | **Selected PI3K pathway combination strategies**

Targets for combination strategy	Tumour stratification	Refs
Tyrosine kinases	Active or overexpressed tyrosine kinase	60,146–151
MEK	Active RTK, RAS mutant	27,156, 157,162
$BRAF^{V600E}$	Melanoma, colon cancer	163,116
MYC	MYC amplification, Notch mutant	28,29, 169–171,173
Autophagy	Glioma, leukaemia, others	174–176
PARP	TNBC	179,180
Aromatase inhibitors	ER-positive	110,187
BCL-2 antagonists	Leukaemia, lymphoma, others	194–196

BCL-2, B cell lymphoma 2; ER, oestrogen receptor; MEK, MAPK/ERK kinase; PARP, poly(ADP-ribose) polymerase; PI3K, phosphoinositide 3-kinase; RTK, receptor tyrosine kinase; TNBC, triple-negative breast cancer.

in the evolution of metastatic tumour cells[159,160]. Active-site mTOR inhibitors decrease the translation of mRNAs encoding proteins that are involved in EMT and prostate cancer invasion[161].

A major concern is whether a therapeutic window can be achieved with combinations of PI3K–AKT–mTOR and MEK inhibitors[162]. To overcome likely toxicities, it might be necessary to experiment with the dose and schedule, such as high-dose intermittent treatments or alternating sequences of doses. Validation of downstream or parallel effectors (such as eIF4E, S6K, MNK, PIM and RSKs) might lead to more tolerable combinations with anticancer efficacy. One setting in which toxicity should be minimized is in colorectal cancers harbouring *$BRAF^{V600E}$*. Selective inhibitors of mutant BRAF (such as vemurafinib) are well tolerated but ineffective owing to compensatory signalling. Combining BRAF inhibitors with a PI3K–mTOR inhibitor caused apoptosis and tumour regression in a model of colorectal cancer driven by mutant BRAF[163]. A recent study showed persistent mTORC1 activity in vemurafinib-resistant melanomas[116], further providing the rationale for combining vemurafinib with mTOR inhibitors in this setting.

The *MYC* oncogene is frequently amplified in cancer and can confer resistance to PI3K–AKT–mTOR inhibitors independently of the RAS pathway[28,29]. Recent breakthroughs indicate that it might be possible to suppress the *MYC* transcriptional programme indirectly by targeting bromo and extra-terminal (BET) proteins such as bromodomain-containing protein 2 (BRD2) and BRD4, which are transcriptional regulators that are required for the efficient expression of MYC[164–167]. Inhibition of the BET–histone interaction by small molecules blocking the bromodomain binding site (so-called BET inhibitors) can downregulate the expression of MYC and its target genes in tumour cells[164–167]. Combining BET inhibitors with PI3K–AKT–mTOR inhibitors is a sensible strategy, particularly in haematopoietic malignancies where the cooperation of MYC with PI3K has been established[168]. *MYC* is the defining oncogene of Burkitt lymphoma, and there is evidence for a cooperative role of MYC and PI3K in patients with Burkitt lymphoma as well as in a mouse model of the disease[169,170]. Inhibiting mTOR or eIF4E strongly impaired MYC-induced lymphomagenesis in mice[171]. In T cell acute lymphoblastic leukaemia (T-ALL), two common lesions are loss of PTEN and activating Notch mutations that elevate MYC activity[172,173]. Hence, either Notch inhibitors or BET inhibitors could be combined with PI3K–AKT–mTOR inhibitors in clinical trials of T-ALL.

Blocking autophagy might provide another avenue to augment cancer cell killing by PI3K–AKT–mTOR inhibitors[174]. Autophagy is a process by which cells recycle organelles and macromolecules to survive under conditions of starvation or other stresses. Inhibition of mTOR causes an autophagy response that is comparable to nutrient starvation. In glioma, leukaemia and other cancer cell types, chemical inhibitors of autophagy potentiate apoptosis induced by active-site mTOR inhibitors or dual PI3K–mTOR inhibitors[175,176]. A combination trial of temsirolimus with an autophagy inhibitor in renal cell carcinoma is underway[174]. However, current autophagy inhibitors are nonspecific agents that generally act by inhibiting lysosomal degradation. The discovery of compounds that inhibit specific components of the autophagy machinery will be helpful for testing the potential of combination approaches with PI3K–AKT–mTOR inhibitors. A related issue is how PI3K–AKT–mTOR inhibitors will affect the response to emerging therapies targeting cancer cell metabolism. Considering that the PI3K–AKT–mTOR pathway drives many of the metabolic hallmarks of cancer cells, it is possible that inhibition of this pathway will reduce sensitivity to metabolic interventions.

Emerging evidence connects the PI3K–AKT–mTOR network to the maintenance of genome integrity. PI3K is involved in sensing double-strand breaks[177,178] and in maintaining the expression of breast cancer susceptibility 1 (BRCA1) and BRCA2, which participate in homologous recombination[179]. Two groups exploited these findings to show that PI3K inhibitors increase DNA damage and sensitize triple-negative breast cancer (TNBC) cells to inhibitors of poly(ADP-ribose) polymerase (PARP)[179,180]. A Phase I trial of the PARP inhibitor olaparib with the pan-PI3K inhibitor BKM120 has been initiated, enrolling patients with TNBC or high-grade serous ovarian cancer.

Paradoxically, PTEN also has a role in protecting cells from genotoxic stress mediated by a nuclear pool of the phosphatase[181]. Elevated PI3K survival signalling in PTEN-deficient cells protects them from accumulated DNA damage. This property renders PTEN-deficient tumours sensitive to the combination of PI3K inhibitors and DNA-damaging agents in preclinical studies[181]. Loss of PTEN might also sensitize tumours to PARP inhibitors, similarly to BRCA1-deficient tumours. It is worth noting that several DNA repair enzymes are members of the PIKK family, including ATM, ATR and DNA-PK[182]. Some of the inhibitors developed against class I PI3Ks have off-target effects on PIKK family

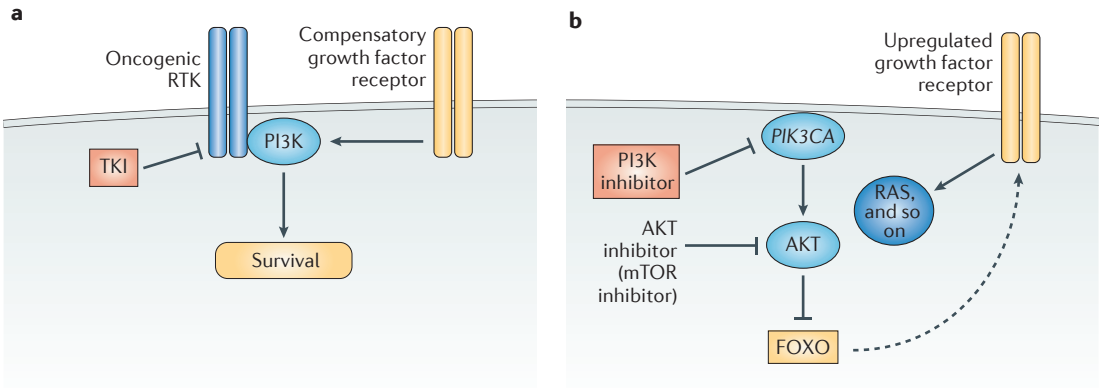


Figure 3 | **Two arguments for combining TKIs with PI3K–AKT–mTOR inhibitors. a** | In cancers that are driven by activated tyrosine kinases, resistance to tyrosine kinase inhibitors (TKIs) can develop through alternative pathways that maintain phosphoinositide 3-kinase (PI3K) signalling, such as compensatory growth factor receptors, loss of phosphatase and tensin homolog (PTEN), mutations in *PIK3CA* (which encodes the PI3K catalytic isoform p110α) or activation of RAS. Combined targeting of PI3K can prevent or overcome drug resistance. **b** | In cancers that are driven by lesions in PI3K or PTEN, inhibiting PI3K, AKT, mammalian target of rapamycin complex 1 (mTORC1) or mTORC2 can cause elevated growth factor receptor signalling through forkhead box O (FOXO)-dependent gene expression. Adding a TKI can ameliorate this compensatory signalling mechanism. RTK, receptor tyrosine kinase.

members, and this property might enhance their synergy with DNA-damaging agents[183]. Inhibiting mTOR can also promote DNA damage through the suppression of Fanconi anaemia group D2 protein (FANCD2) and other mechanisms[184–186]. Thus, an important area for continuing study is to investigate how inhibitors acting at different levels of the PI3K–AKT–mTOR network affect the cellular response to radiation and chemotherapeutic drugs, which are currently the standard of care in many cancers. It is also worth considering that the off-target effects on DNA-PK and ATM are probably a liability rather than an advantage, if they are not combined with DNA-damaging agents or radiation, as they increase genomic instability, which tends to accelerate drug resistance.

The BOLERO-2 trial illustrated the potential of PI3K–AKT–mTOR inhibitors to prevent or overcome targeted therapy in hormone-dependent cancers. There is evidence for positive feedback between hormone receptors and the PI3K network[187]. The ligand-bound oestrogen receptor (ER) interacts directly with PI3K, augmenting PI3K–AKT activity. In turn, AKT and S6K1 can phosphorylate hormone receptors to increase its activity. Hormone-dependent cancers frequently exhibit high basal PI3K activity through the loss of PTEN, *PIK3CA* mutations or other mechanisms[188,189]. Therefore, inhibitors acting at multiple levels of the PI3K–AKT–mTOR network might supplement the anticancer effects of hormone therapy. In advanced prostate cancer with loss of PTEN, it would be interesting to test a p110β inhibitor in combination with an androgen receptor antagonist. There is also a rationale for a dual p110β–p110δ inhibitor in this setting, based on a report that B cell infiltrates sustain prostate cancer survival after hormone withdrawal[190]. This strategy would act via tumour-intrinsic effects (p110β) together with extrinsic effects on immune infiltration (p110δ).

A conceptually simple approach for sensitizing cancer cells to PI3K pathway-targeted agents is to combine these drugs with agents that increase mitochondrial priming for death. B cell lymphoma 2 (BCL-2) family members (BCL-2, BCL-X_L, myeloid cell leukaemia sequence 1 (MCL1) and BCL-2-related protein A1) maintain mitochondrial integrity by blocking the pro-apoptotic function of BCL-2-associated X protein (BAX) and BCL-2 antagonist/killer (BAK)[191]. A large family of pro-apoptotic proteins that are homologous to BCL-2 can sequester the pro-survival proteins or directly activate BAX and BAK[192]. Priming refers to suppression of the activity of pro-survival factors at the mitochondria, such as BCL-2 and MCL1, relative to pro-apoptotic proteins such as BIM and PUMA[193]. ABT-263, a small-molecule inhibitor of BCL-2 and BCL-X_L, has entered clinical trials for cancer and shown some promise in CLL[191]. By increasing mitochondrial priming, BCL-2 antagonists should lower the threshold for apoptosis in response to PI3K–AKT–mTOR inhibition (FIG. 4). A growing body of work supports the synergistic antitumour effects of PI3K–AKT–mTOR inhibitors combined with BCL-2 antagonists[157,194–196].

Future directions

Building on the discussions above, we envision four key strategies that will maximize the potential of PI3K–AKT–mTOR inhibitors in oncology.

Biomarker identification through next-generation sequencing. A limited response rate with a single-agent strategy at an early stage of development does not necessarily mean that a clinical trial has failed, especially when modulating a genetically validated target or disease biology. The advent of next-generation sequencing allows considerable knowledge to be gained from the rare responders in a trial. The '$n=1$ response' matters. An exciting report

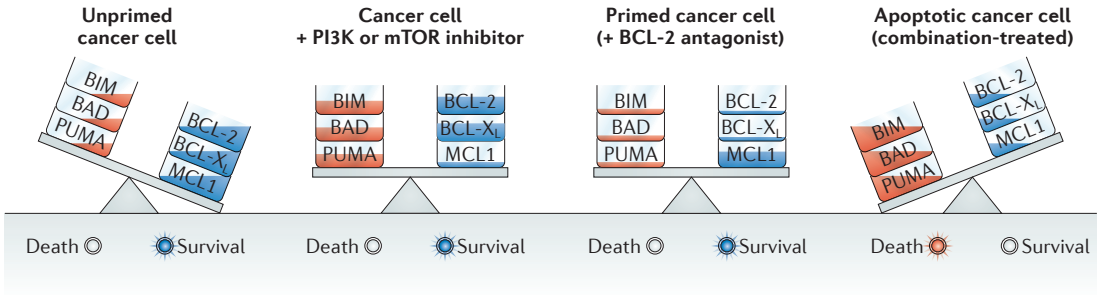


Figure 4 | **Rationale for BCL-2 antagonist combinations.** The balance of pro-survival and pro-apoptotic B cell lymphoma 2 (BCL-2) family members at the mitochondria is a primary factor controlling cell survival versus apoptosis. Phosphoinositide 3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) signalling suppresses the expression and activity of multiple pro-apoptotic proteins (that is, BCL-2 antagonist of cell death (BAD), BCL-2-interacting mediator of cell death (BIM), p53-upregulated modulator of apoptosis (PUMA) and death receptors) and can increase the expression of pro-survival factors (such as myeloid cell leukaemia sequence 1 (MCL1)). However, PI3K–AKT–mTOR inhibition does not necessarily tip the balance towards apoptosis. Small-molecule antagonists of pro-survival proteins (such as BCL-2 and BCL-X_L) increase mitochondrial 'priming' for death, lowering the threshold for the induction of apoptosis by PI3K–AKT–mTOR inhibitors.

from Solit and colleagues[113] used exome sequencing to identify *TSC1* inactivation in a rare bladder cancer that responded to everolimus. Targeted sequencing of additional tumours from clinical trials of everolimus showed a statistically significant delay in recurrence for samples with *TSC1* mutations[102]. As sequencing costs decline and technologies improve, it should be feasible to apply this approach in clinical trials of PI3K–AKT–mTOR inhibitors as single agents or in combinations. This idea should not replace patient selection based on the drug target, cancer genetics and disease biology. It should, however, be applied in parallel to select additional genetic markers for subsequent trials.

Initial emphasis on haematological malignancies. Leukaemia, lymphoma and myeloma are a diverse set of cancers that are individually less common than solid tumours such as lung, breast and colon cancer. Few blood cancers carry activating mutations in RAS or PI3K. Nevertheless, there are several reasons for devoting resources to clinical trials of PI3K–mTOR inhibitors for blood cancers. First, leukaemia and lymphoma models often show non-oncogene addiction to mTOR[60,171]. Second, blood cancers generally express p110δ and/or p110γ, which should, in principle, confer responsiveness to agents targeting these isoforms, as illustrated by GS-1101. Third, unlike many solid tumours, haematopoietic cancer cells are in constant contact with the immune system and might be especially sensitive to the immune-enhancing effects of PI3K–mTOR inhibitors. It is also easier to access tumour cells for pharmacodynamic monitoring in patients with blood cancers, and plasma analysis can provide useful information about immune-modulation[197]. Last, treating rare blood cancers effectively can be rewarding, as proven by BCR–ABL inhibitors that have saved the lives of an ever-expanding population of patients with chronic myeloid leukaemia who must receive long-term therapy.

Harnessing immune effects. Although cancer is a genetic disease of aberrant cells, it is also a chronic immune disease[35,198] (FIG. 1). The immune system restrains tumorigenesis but eventually the tumour enforces a state of immune tolerance and exhaustion. Recently, there has been exciting progress in treating human tumours with immunotherapies to overcome tolerance and exhaustion[35,36,199]. There is also an increasing appreciation of how small molecules targeting the cancer cell affect the immune context of the tumour[200,201]. The efficacy of GS-1101 emphasizes how a drug targeting both the tumour and the immune system can act as an all-in-one combination therapy. How is it possible to harness antitumour immunity through a pathway that is defined by a target of the immunosuppressive drug rapamycin?

Extensive studies of the PI3K–AKT–mTOR network in immune cells have shown that PI3K activation is not a simple 'on/off' switch[117,118,202,203]. Inhibiting the pathway can either suppress or enhance immune responses through its effects on diverse subsets of innate and adaptive immune cells. In theory, it should be possible to implement treatment regimens that increase the immune rejection of tumours in concert with having direct antitumour effects. A key factor limiting progress in this area is that preclinical drug development programmes mainly use tumour xenograft models. These models are convenient and useful for assessing drug pharmacology, providing valuable information about pharmacodynamics in the context of pharmacokinetics and general tolerability, but they overlook any modulation of adaptive immunity, as growing human tumour cells in mice requires host strains that lack functional lymphocytes. Interactions of xenograft cells with components of innate immunity might also fail to recapitulate the events that lead to the development of human tumours. For these reasons, it is essential to test candidate inhibitors in genetically engineered mouse models and to extensively monitor the infiltration and activity of diverse immune subsets,

including macrophages, T cells and natural killer cells. Such systems would be useful for testing ideas such as 'dialing in' activity against p110δ and/or p110γ to create a more favourable immune environment. One can even imagine that inhibiting p110δ and/or p110γ alone in solid tumours would provide significant therapeutic benefit and tolerability without having any direct effect on the PI3K isoforms expressed within the cancer cell (K. Ali, K. Okkenhaug and B. Vanhaesebroeck, personal communication).

It will also be crucial to determine which agents targeting the PI3K–AKT–mTOR pathway enhance or suppress the efficacy of emerging immunotherapies and cancer vaccines. In mouse models, inhibitors of both PI3K and mTOR can enhance the efficacy of immune-directed therapies[204–206]. It is relevant to consider that in contrast to pan-PI3K class I inhibitors, isoform-selective agents minimize immunosuppressive effects on lymphocytes[93]. Hence, pan-PI3K inhibitors are more likely to enforce or accelerate the immune exhaustion state. Eventually, the best combination therapies might turn out to be isoform-selective PI3K or mTOR inhibitors combined with immunotherapies or cancer vaccines. Matching patients to the right combinations will require knowledge of the genomic driver and the immune fingerprint of the tumour.

Combination trials. Above, we proposed several rational combinations to increase the killing of cancer cells by PI3K–AKT–mTOR inhibitors. However, in drug development there is always a degree of conflict between what should be done and what can be done. Developing combination therapies costs more resources and time, and might ultimately result in challenges for reimbursement. Conversely, the experience with BRAF inhibitors shows that combinations will be justified even for therapies that provide an impressive initial response in selected patients. In the short term, the plan should be to prioritize approaches based on feasibility, pragmatism and the likelihood of achieving a meaningful therapeutic advancement. It makes sense to start by combining PI3K–AKT–mTOR inhibitors with approved targeted agents that are the standard of care for specific malignancies. Using a companion drug with a safety profile and optimal dosing that is well understood will reduce the complexity of the combination trial. It will also facilitate the incorporation of PI3K–AKT–mTOR inhibitors into treatment regimens at earlier stages of the disease, rather than only in patients with relapsed or refractory tumours. The limited success of single agents to date might be explained in part by the polygenic and polyclonal nature of advanced tumours, some of which is caused by prior therapies. Preclinical data support the testing of several combinations with approved tyrosine kinase inhibitors: BCR–ABL inhibitors in Philadelphia chromosome-positive (Ph^+) leukaemias and GISTs[149], EGFR inhibitors in lung and colon cancer[146,147], and agents targeting HER2 or ERBB3 in breast cancer[207]. Combining a PI3K inhibitor with a BRAF inhibitor might enhance efficacy in melanoma and produce responses in colorectal cancers expressing mutant BRAF[163,208,209].

Key biological insights from preclinical data can sometimes justify combination trials of two experimental agents. Agents that show synthetic lethality with PI3K pathway inhibitors in cancer cell lines and patient-derived xenografts, but not in normal cells, should be given priority for clinical testing. An example discussed above is the combination of PI3K and PARP inhibitors for TNBC (TABLE 2), in which trials were quickly initiated after remarkable preclinical results were obtained[179,180].

In cases where genetically engineered mouse models exist for driver oncogenes and tumour types, it should be possible to design synchronous 'co-clinical' trials that help in identifying genetic and pharmacodynamic markers of responsiveness[210]. These are especially powerful when paired with studies using patient-derived primary tumour tissue analysis. The regulatory approval of immunotherapies in certain cancers also sets the stage for testing the immune-enhancing potential of PI3K or mTOR inhibitors.

Conclusions

The rationale for targeting the PI3K–AKT–mTOR network in cancer remains anchored on a solid foundation of cancer genetics and cell biology studies. Despite many challenges, measurable advances have been made in the clinic. Rapalogues are useful in some advanced cancers and as adjuvants to hormone therapy in breast cancer. Inhibitors of PI3Kδ and BTK are on track for FDA approval in certain B cell malignancies. Other agents are advancing through development. Nevertheless, early hopes have been tempered by the realization that targeting the PI3K–AKT–mTOR pathway alone will not be a cure-all for diverse cancers.

How can we reset strategies to maximize the potential of PI3K–AKT–mTOR inhibitors? Previous experiences with successful oncology drug development show the importance of three factors: targeting genetic drivers in selected patient populations; understanding the biology of crosstalk and feedback to use effective combinations; and stimulating an immune environment that favours tumour eradication. Thoughtful application of these principles will light the path towards effective cancer control by PI3K–AKT–mTOR inhibitors.

1. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
2. Beagle, B. & Fruman, D. A. A lipid kinase cousin cooperates to promote cancer. *Cancer Cell* **19**, 693–695 (2011).
3. Soler, A. *et al.* Inhibition of the p110α isoform of PI3-kinase stimulates nonfunctional tumor angiogenesis. *J. Exp. Med.* **210**, 1937–1945 (2013).
4. Hirsch, E., Ciraolo, E., Franco, I., Ghigo, A. & Martini, M. PI3K in cancer–stroma interactions: bad in seed and ugly in soil. *Oncogene* http://dx.doi.org/10.1038/onc.2013.265 (2013).
5. Vanhaesebroeck, B. *et al.* Synthesis and function of 3-phosphorylated inositol lipids. *Annu. Rev. Biochem.* **70**, 535–602 (2001).
6. Zhao, J. J. & Roberts, T. M. PI3 kinases in cancer: from oncogene artifact to leading cancer target. *Sci. STKE* **2006**, e52 (2006).
7. Lemmon, M. A. Membrane recognition by phospholipid-binding domains. *Nature Rev. Mol. Cell Biol.* **9**, 99–111 (2008).
8. Samuels, Y. & Ericson, K. Oncogenic PI3K and its role in cancer. *Curr. Opin. Oncol.* **18**, 77–82 (2006).

9. Song, M. S., Salmena, L. & Pandolfi, P. P. The functions and regulation of the PTEN tumour suppressor. *Nature Rev. Mol. Cell Biol.* **13**, 283–296 (2012).
10. Lui, V. W. *et al.* Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. *Cancer Discov.* **3**, 761–769 (2013).
11. Engelman, J. A. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nature Rev. Cancer* **9**, 550–562 (2009).
12. Berndt, A. *et al.* The p110δ structure: mechanisms for selectivity and potency of new PI(3)K inhibitors. *Nature Chem. Biol.* **6**, 244 (2010).
13. Huang, C. H. *et al.* The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kα mutations. *Science* **318**, 1744–1748 (2007).
14. Miled, N. *et al.* Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science* **317**, 239–242 (2007).
References 13 and 14 are two hallmark structural studies of p110α.
15. Vadas, O., Burke, J. E., Zhang, X., Berndt, A. & Williams, R. L. Structural basis for activation and inhibition of class I phosphoinositide 3-kinases. *Sci. Signal.* **4**, re2 (2011).
16. Walker, E. H., Perisic, O., Ried, C., Stephens, L. & Williams, R. L. Structural insights into phosphoinositide 3-kinase catalysis and signalling. *Nature* **402**, 313–320 (1999).
This paper reports first X-ray structure of a class I PI3K.
17. Wu, H. *et al.* Regulation of Class IA PI 3-kinases: C2 domain-iSH2 domain contacts inhibit p85/p110alpha and are disrupted in oncogenic p85 mutants. *Proc. Natl Acad. Sci. USA* **106**, 20258–20263 (2009).
18. Zhang, X. *et al.* Structure of lipid kinase p110β/p85β elucidates an unusual SH2-domain-mediated inhibitory mechanism. *Mol. Cell* **41**, 567–578 (2011).
19. Garcia-Echeverria, C. & Sellers, W. R. Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene* **27**, 5511–5526 (2008).
20. Wander, S. A., Hennessy, B. T. & Slingerland, J. M. Next-generation mTOR inhibitors in clinical oncology: how pathway complexity informs therapeutic strategy. *J. Clin. Invest.* **121**, 1231–1241 (2011).
21. Workman, P., Clarke, P. A., Raynaud, F. I. & van Montfort, R. L. Drugging the PI3 kinome: from chemical tools to drugs in the clinic. *Cancer Res.* **70**, 2146–2157 (2010).
22. Agarwal, R., Carey, M., Hennessy, B. & Mills, G. B. PI3K pathway-directed therapeutic strategies in cancer. *Curr. Opin. Investigat. Drugs* **11**, 615–628 (2010).
23. Marone, R., Cmiljanovic, V., Giese, B. & Wymann, M. P. Targeting phosphoinositide 3-kinase: moving towards therapy. *Biochim. Biophys. Acta* **1784**, 159–185 (2008).
24. Yap, T. A. *et al.* Targeting the PI3K–AKT–mTOR pathway: progress, pitfalls, and promises. *Curr. Opin. Pharmacol.* **8**, 393–412 (2008).
25. Rodon, J., Dienstmann, R., Serra, V. & Tabernero, J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nature Rev. Clin. Oncol.* **10**, 143–153 (2013).
26. Klempner, S. J., Myers, A. P. & Cantley, L. C. What a tangled web we weave: emerging resistance mechanisms to inhibition of the phosphoinositide 3-kinase pathway. *Cancer Discov.* **3**, 1345–1354 (2013).
27. Engelman, J. A. *et al.* Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nature Med.* **14**, 1351–1356 (2008).
This paper provides the first proof of concept *in vivo* for co-targeting PI3K and MEK.
28. Ilic, N., Utermark, T., Widlund, H. R. & Roberts, T. M. PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. *Proc. Natl Acad. Sci. USA* **108**, E699–E708 (2011).
29. Liu, P. *et al.* Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. *Nature Med.* **17**, 1116–1120 (2011).
This reversible *PIK3CA* model showed mechanisms of relapse.
30. Kinross, K. M. *et al.* An activating *Pik3ca* mutation coupled with *Pten* loss is sufficient to initiate ovarian tumorigenesis in mice. *J. Clin. Invest.* **122**, 553–557 (2012).
31. Tikoo, A. *et al.* Physiological levels of Pik3ca(H1047R) mutation in the mouse mammary gland results in ductal hyperplasia and formation of ERalpha-positive tumors. *PLoS ONE* **7**, e36924 (2012).
32. Fruman, D. A. & Rommel, C. PI3Kδ inhibitors in cancer: rationale and serendipity merge in the clinic. *Cancer Discov.* **1**, 562–572 (2011).
33. Macias-Perez, I. M. & Flinn, I. W. GS-1101: a delta-specific PI3K inhibitor in chronic lymphocytic leukemia. *Curr. Hematol. Malignancy Rep.* **8**, 22–27 (2013).
34. Burger, J. A. Targeting the microenvironment in chronic lymphocytic leukemia is changing the therapeutic landscape. *Curr. Opin. Oncol.* **24**, 643–649 (2012).
35. Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1–10 (2013).
36. Riley, J. L. Combination checkpoint blockade — taking melanoma immunotherapy to the next level. *N. Engl. J. Med.* **369**, 187–189 (2013).
37. Salmena, L., Carracedo, A. & Pandolfi, P. P. Tenets of PTEN tumor suppression. *Cell* **133**, 403–414 (2008).
38. Vanhaesebroeck, B., Guillermet-Guibert, J., Graupera, M. & Bilanges, B. The emerging mechanisms of isoform-specific PI3K signalling. *Nature Rev. Mol. Cell Biol.* **11**, 329–341 (2010).
39. Fritsch, R. *et al.* RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms. *Cell* **153**, 1050–1063 (2013).
This paper reports the discovery that RAC and CDC42, and not RAS, contribute to the activation of p110β.
40. Fruman, D. A. Towards an understanding of isoform specificity in phosphoinositide 3-kinase signalling in lymphocytes. *Biochem. Soc. Trans.* **32**, 315–319 (2004).
41. Hawkins, P. T., Stephens, L. R., Suire, S. & Wilson, M. PI3K signaling in neutrophils. *Curr. Top. Microbiol. Immunol.* **346**, 183–202 (2010).
42. Okkenhaug, K., Ali, K. & Vanhaesebroeck, B. Antigen receptor signalling: a distinctive role for the p110δ isoform of PI3K. *Trends Immunol.* **28**, 80–87 (2007).
43. Okkenhaug, K. & Fruman, D. A. PI3Ks in lymphocyte signaling and development. *Curr. Top. Microbiol. Immunol.* **346**, 57–85 (2011).
44. Foukas, L. C., Berenjeno, I. M., Gray, A., Khwaja, A. & Vanhaesebroeck, B. Activity of any class IA PI3K isoform can sustain cell proliferation and survival. *Proc. Natl Acad. Sci. USA* **107**, 11381–11386 (2010).
This paper provides evidence for the redundant functions of PI3K isoforms in cell proliferation and survival.
45. Dbouk, H. A. *et al.* Characterization of a tumor-associated activating mutation of the p110β PI 3-kinase. *PLoS ONE* **8**, e63833 (2013).
46. Cheung, L. W. *et al.* High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. *Cancer Discov.* **1**, 170–185 (2011).
47. Jaiswal, B. S. *et al.* Somatic mutations in p85α promote tumorigenesis through class IA PI3K activation. *Cancer Cell* **16**, 463–474 (2009).
48. Sun, M., Hillmann, P., Hofmann, B. T., Hart, J. R. & Vogt, P. K. Cancer-derived mutations in the regulatory subunit p85α of phosphoinositide 3-kinase function through the catalytic subunit p110α. *Proc. Natl Acad. Sci. USA* **107**, 15547–15552 (2010).
49. Wee, S. *et al.* PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. *Cancer Res.* **69**, 4286–4293 (2009).
50. Ludovini, V. *et al.* Phosphoinositide-3-kinase catalytic alpha and KRAS mutations are important predictors of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in patients with advanced non-small cell lung cancer. *J. Thorac. Oncol.* **6**, 707–715 (2011).
51. Suda, K., Mizuuchi, H., Maehara, Y. & Mitsudomi, T. Acquired resistance mechanisms to tyrosine kinase inhibitors in lung cancer with activating epidermal growth factor receptor mutation — diversity, ductility, and destiny. *Cancer Metastasis Rev.* **31**, 807–814 (2012).
52. Cybulski, N. & Hall, M. N. TOR complex 2: a signaling pathway of its own. *Trends Biochem. Sci.* **34**, 620–627 (2009).
53. Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. *Cell* **149**, 274–293 (2012).
54. Zinzalla, V., Stracka, D., Oppliger, W. & Hall, M. N. Activation of mTORC2 by association with the ribosome. *Cell* **144**, 757–768 (2011).
55. Huang, J. & Manning, B. D. The TSC1–TSC2 complex: a molecular switchboard controlling cell growth. *Biochem. J.* **412**, 179–190 (2008).
56. Dibble, C. C. *et al.* TBC1D7 is a third subunit of the TSC1–TSC2 complex upstream of mTORC1. *Mol. Cell* **47**, 535–546 (2012).
57. Guertin, D. A. *et al.* mTOR complex 2 is required for the development of prostate cancer induced by *Pten* loss in mice. *Cancer Cell* **15**, 148–159 (2009).
58. Nardella, C. *et al.* Differential requirement of mTOR in postmitotic tissues and tumorigenesis. *Sci. Signal.* **2**, ra2 (2009).
References 57 and 58 genetically validate mTOR as a selective cancer target in prostate cancer.
59. Evangelisti, C. *et al.* Targeted inhibition of mTORC1 and mTORC2 by active-site mTOR inhibitors has cytotoxic effects in T-cell acute lymphoblastic leukemia. *Leukemia* **25**, 781–791 (2011).
60. Janes, M. R. *et al.* Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. *Nature Med.* **16**, 205–213 (2010).
61. Chandarlapaty, S. *et al.* AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer Cell* **19**, 58–71 (2011).
62. Rodrik-Outmezguine, V. S. *et al.* mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discov.* **1**, 248–259 (2011).
This is a detailed analysis of the feedback effects of mTOR kinase inhibitors and the role of FOXO transcription factors.
63. Ballif, B. A. *et al.* Quantitative phosphorylation profiling of the ERK/p90 ribosomal S6 kinase-signaling cassette and its targets, the tuberous sclerosis tumor suppressors. *Proc. Natl Acad. Sci. USA* **102**, 667–672 (2005).
64. Ma, L., Chen, Z., Erdjument-Bromage, H., Tempst, P. & Pandolfi, P. P. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* **121**, 179–193 (2005).
65. Ma, L. *et al.* Identification of S664 TSC2 phosphorylation as a marker for extracellular signal-regulated kinase mediated mTOR activation in tuberous sclerosis and human cancer. *Cancer Res.* **67**, 7106–7112 (2007).
66. Tabernero, J. *et al.* Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. *J. Clin. Oncol.* **26**, 1603–1610 (2008).
67. She, Q. B. *et al.* 4E-BP1 is a key effector of the oncogenic activation of the AKT and ERK signaling pathways that integrates their function in tumors. *Cancer Cell* **18**, 39–51 (2010).
This paper provides evidence for the convergence of PI3K–AKT and RAS–ERK signals at the level of 4EBPs.
68. Wang, X. *et al.* Inhibition of mammalian target of rapamycin induces phosphatidylinositol 3-kinase-dependent and Mnk-mediated eukaryotic translation initiation factor 4E phosphorylation. *Mol. Cell. Biol.* **27**, 7405–7413 (2007).
69. Lee, T., Yao, G., Nevins, J. & You, L. Sensing and integration of Erk and PI3K signals by Myc. *PLoS Computat. Biol.* **4**, e1000013 (2008).
70. Brachmann, S. M. *et al.* Characterization of the mechanism of action of the pan class I PI3K inhibitor NVP-BKM120 across a broad range of concentrations. *Mol. Cancer Ther.* **11**, 1747–1757 (2012).
71. Advani, R. H. *et al.* Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J. Clin. Oncol.* **31**, 88–94 (2013).
72. Byrd, J. C. *et al.* Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N. Engl. J. Med.* **369**, 32–42 (2013).
73. Corcoran, R. B. *et al.* EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov.* **2**, 227–235 (2012).
74. Prahallad, A. *et al.* Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* **483**, 100–103 (2012).

75. Garrett, J. T. *et al.* Combination of antibody that inhibits ligand-independent HER3 dimerization and a p110α inhibitor potently blocks PI3K signaling and growth of HER2$^+$ breast cancers. *Cancer Res.* **73**, 6013–6023 (2013).
76. Gupta, S. *et al.* Binding of Ras to phosphoinositide 3-kinase p110α is required for Ras-driven tumorigenesis in mice. *Cell* **129**, 957–968 (2007). **This knock-in mouse defined a role for p110α in RAS transformation.**
77. Nacht, M. *et al.* Discovery of a potent and isoform-selective targeted covalent inhibitor of the lipid kinase PI3Kα. *J. Med. Chem.* **56**, 712–721 (2013).
78. Lee, J. H. *et al. De novo* somatic mutations in components of the PI3K–AKT3–mTOR pathway cause hemimegalencephaly. *Nature Genet.* **44**, 941–945 (2012).
79. Lindhurst, M. J. *et al.* Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in PIK3CA. *Nature Genet.* **44**, 928–933 (2012).
80. Riviere, J. B. *et al. De novo* germline and postzygotic mutations in *AKT3*, *PIK3R2* and *PIK3CA* cause a spectrum of related megalencephaly syndromes. *Nature Genet.* **44**, 934–940 (2012).
81. Angulo, I. *et al.* Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. *Science* **342**, 866–871 (2013).
82. Lucas, C. L. *et al.* Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nature Immunol.* http://dx.doi.org/10.1038/ni.2771 (2013). **References 81 and 82 identify human immunodeficiency patients with gain-of-function mutations affecting p110δ.**
83. Jia, S. *et al.* Essential roles of PI(3)K-p110β in cell growth, metabolism and tumorigenesis. *Nature* **454**, 776–779 (2008). **This paper provides the first genetic evidence for p110β function in tumorigenesis.**
84. Torbett, N. E. *et al.* A chemical screen in diverse breast cancer cell lines reveals genetic enhancers and suppressors of sensitivity to PI3K isoform-selective inhibition. *Biochem. J.* **415**, 97–110 (2008).
85. Wee, S. *et al.* PTEN-deficient cancers depend on PIK3CB. *Proc. Natl Acad. Sci. USA* **105**, 13057–13062 (2008).
86. Berenjeno, I. M. *et al.* Both p110α and p110β isoforms of PI3K can modulate the impact of loss-of-function of the PTEN tumour suppressor. *Biochem. J.* **442**, 151–159 (2012).
87. Iyengar, S. *et al.* P110α-mediated constitutive PI3K signaling limits the efficacy of p110δ-selective inhibition in mantle cell lymphoma, particularly with multiple relapse. *Blood* **121**, 2274–2284 (2013).
88. Liu, N. *et al.* BAY 80–6946 is a highly selective intravenous PI3K inhibitor with potent p110α and p110δ activities in tumor cell lines and xenograft models. *Mol. Cancer Ther.* **12**, 2319–2330 (2013).
89. Subramaniam, P. S. *et al.* Targeting nonclassical oncogenes for therapy in T-ALL. *Cancer Cell* **21**, 459–472 (2012). **This paper provides proof of concept for the dual targeting of p110γ and p110δ in T cell leukaemia.**
90. Winkler, D. G. *et al.* PI3K-δ and PI3K-γ inhibition by IPI-145 abrogates immune responses and suppresses activity in autoimmune and inflammatory disease models. *Chem. Biol.* **20**, 1364–1374 (2013).
91. Boyle, D. L. Kim, H. R., Topolewski, K., Bartok, B. & Firestein, G. S. Novel dual phosphoinositide 3-kinase-δ,γ inhibitor: potent anti-inflammatory effects and joint protection in models of rheumatoid arthritis. *J. Pharmacol. Exp. Ther.* http://dx.doi.org/10.1124/jpet.113.205955 (2013).
92. Schmid, M. C. *et al.* Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3kγ, a single convergent point promoting tumor inflammation and progression. *Cancer Cell* **19**, 715–727 (2011). **This study shows that p110γ activity in myeloid cells acts downstream of diverse receptors and promotes the formation of solid tumours even though the isoform is not expressed in cancer cells.**
93. So, L. *et al.* Selective inhibition of phosphoinositide 3-kinase p110α preserves lymphocyte function. *J. Biol. Chem.* **288**, 5718–5731 (2013).
94. Brunn, G. J. *et al.* Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *EMBO J.* **15**, 5256–5267 (1996).
95. Gharbi, S. I. *et al.* Exploring the specificity of the PI3K family inhibitor LY294002. *Biochem. J.* **404**, 15–21 (2007).
96. Knight, Z. A. *et al.* A pharmacological map of the PI3-K family defines a role for p110α in insulin signaling. *Cell* **125**, 733–747 (2006).
97. Kharas, M. G. *et al.* Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR-ABL$^+$ leukemia cells. *J. Clin. Invest.* **118**, 3038–3050 (2008).
98. Flaherty, K. T. *et al.* Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N. Engl. J. Med.* **367**, 1694–1703 (2012).
99. Elkabets, M. *et al.* mTORC1 inhibition is required for sensitivity to PI3K p110α inhibitors in PIK3CA-mutant breast cancer. *Sci. Transl. Med.* **5**, 196ra99 (2013). **This study demonstrates that mTORC1 preserves survival in *PIK3CA*-mutant cells treated with p110α inhibitors.**
100. Yuan, R., Kay, A., Berg, W. J. & Lebwohl, D. Targeting tumorigenesis: development and use of mTOR inhibitors in cancer therapy. *J. Hematol. Oncol.* **2**, 45 (2009).
101. Sankhala, K. *et al.* The emerging safety profile of mTOR inhibitors, a novel class of anticancer agents. *Target Oncol.* **4**, 135–142 (2009).
102. Benjamin, D., Colombi, M., Moroni, C. & Hall, M. N. Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nature Rev. Drug Discov.* **10**, 868–880 (2011).
103. Janes, M. R. & Fruman, D. A. Targeting TOR dependence in cancer. *Oncotarget* **1**, 69–76 (2010).
104. Gentzler, R. D., Altman, J. K. & Platanias, L. C. An overview of the mTOR pathway as a target in cancer therapy. *Expert Opin. Ther. Targets* **16**, 481–489 (2012).
105. Chresta, C. M. *et al.* AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with *in vitro* and *in vivo* antitumor activity. *Cancer Res.* **70**, 288–298 (2010).
106. Yu, K. *et al.* Beyond rapalog therapy: preclinical pharmacology and antitumor activity of WYE-125132, an ATP-competitive and specific inhibitor of mTORC1 and mTORC2. *Cancer Res.* **70**, 621–631 (2010).
107. Garcia-Garcia, C. *et al.* Dual mTORC1/2 and HER2 blockade results in antitumor activity in preclinical models of breast cancer resistant to anti-HER2 therapy. *Clin. Cancer Res.* **18**, 2603–2612 (2012).
108. Alain, T., Sonenberg, N. & Topisirovic, I. mTOR inhibitor efficacy is determined by the eIF4E/4E-BP ratio. *Oncotarget* **3**, 1491–1492 (2012).
109. Martineau, Y. *et al.* Pancreatic tumours escape from translational control through 4E-BP1 loss. *Oncogene* http://dx.doi.org/10.1038/onc.2013.100 (2013).
110. Baselga, J. *et al.* Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N. Engl. J. Med.* **366**, 520–529 (2012). **This cinical study establishes the combination of rapalogues with anti-oestrogen therapy in breast cancer.**
111. Bissler, J. J. *et al.* Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangio-leiomyomatosis. *N. Engl. J. Med.* **358**, 140–151 (2008).
112. Krueger, D. A. *et al.* Everolimus long-term safety and efficacy in subependymal giant cell astrocytoma. *Neurology* **80**, 574–580 (2013).
113. Iyer, G. *et al.* Genome sequencing identifies a basis for everolimus sensitivity. *Science* **338**, 221 (2012). **This paper demonstrates that genome sequencing of rare responders can identify predictive biomarkers for rapalogue sensitivity.**
114. Bar-Peled, L. *et al.* A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science* **340**, 1100–1106 (2013).
115. Panchaud, N., Peli-Gulli, M. P. & De Virgilio, C. Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1. *Sci. Signal.* **6**, ra42 (2013).
116. Corcoran, R. B. *et al.* TORC1 suppression predicts responsiveness to RAF and MEK inhibition in BRAF-mutant melanoma. *Sci. Transl. Med.* **5**, 196ra98 (2013).
117. Powell, J. D., Pollizzi, K. N., Heikamp, E. B. & Horton, M. R. Regulation of immune responses by mTOR. *Annu. Rev. Immunol.* **30**, 39–68 (2012).
118. Thomson, A. W., Turnquist, H. R. & Raimondi, G. Immunoregulatory functions of mTOR inhibition. *Nature Rev. Immunol.* **9**, 324–337 (2009).
119. Zeng, H. & Chi, H. mTOR and lymphocyte metabolism. *Curr. Opin. Immunol.* **25**, 347–355 (2013).
120. Araki, K. *et al.* mTOR regulates memory CD8 T-cell differentiation. *Nature* **460**, 108–112 (2009).
121. Delgoffe, G. M. *et al.* The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* **30**, 832–844 (2009).
122. Delgoffe, G. M. *et al.* The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nature Immunol.* **12**, 295–303 (2011). **This is an informative dissection of the functions of mTORC1 and mTORC2 in T cell differentiation, which were determined using genetic and pharmacological approaches.**
123. Katholnig, K., Linke, M., Pham, H., Hengstschlager, M. & Weichhart, T. Immune responses of macrophages and dendritic cells regulated by mTOR signalling. *Biochem. Soc. Trans.* **41**, 927–933 (2013).
124. Procaccini, C. *et al.* An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. *Immunity* **33**, 929–941 (2010).
125. Zeng, H. *et al.* mTORC1 couples immune signals and metabolic programming to establish T_{reg}-cell function. *Nature* **499**, 485–490 (2013).
126. Engelman, J. A., Luo, J. & Cantley, L. C. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nature Rev. Genet.* **7**, 619 (2006).
127. Wullschleger, S., Loewith, R. & Hall, M. N. TOR signaling in growth and metabolism. *Cell* **124**, 471–484 (2006).
128. Bellacosa, A., Testa, J. R., Staal, S. P. & Tsichlis, P. N. A retroviral oncogene, *akt*, encoding a serine-threonine kinase containing an SH2-like region. *Science* **254**, 274–277 (1991).
129. Rhodes, N. *et al.* Characterization of an Akt kinase inhibitor with potent pharmacodynamic and antitumor activity. *Cancer Res.* **68**, 2366–2374 (2008).
130. Yap, T. A. *et al.* First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. *J. Clin. Oncol.* **29**, 4688–4695 (2011).
131. Cho, H. *et al.* Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBβ). *Science* **292**, 1728–1731 (2001).
132. Pal, S. K., Reckamp, K., Yu, H. & Figlin, R. A. Akt inhibitors in clinical development for the treatment of cancer. *Expert Opin. Investigat. Drugs* **19**, 1355–1366 (2010).
133. Lin, J. *et al.* Targeting activated Akt with GDC-0068, a novel selective Akt inhibitor that is efficacious in multiple tumor models. *Clin. Cancer Res.* **19**, 1760–1772 (2013).
134. Vakana, E., Altman, J. K. & Platanias, L. C. Targeting AMPK in the treatment of malignancies. *J. Cell. Biochem.* **113**, 404–409 (2012).
135. Lindqvist, L. & Pelletier, J. Inhibitors of translation initiation as cancer therapeutics. *Future Med. Chem.* **1**, 1709–1722 (2009).
136. Moerke, N. J. *et al.* Small-molecule inhibition of the interaction between the translation initiation factors eIF4E and eIF4G. *Cell* **128**, 257–267 (2007).
137. Li, S., Brown, M. S. & Goldstein, J. L. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc. Natl Acad. Sci. USA* **107**, 3441–3446 (2010).
138. Okuzumi, T. *et al.* Inhibitor hijacking of Akt activation. *Nature Chem. Biol.* **5**, 484–493 (2009).
139. Pearce, L. R. *et al.* Characterization of PF-4708671, a novel and highly specific inhibitor of p70 ribosomal S6 kinase (S6K1). *Biochem. J.* **431**, 245–255 (2010).
140. Tandon, P. *et al.* Requirement for ribosomal protein S6 kinase 1 to mediate glycolysis and apoptosis resistance induced by Pten deficiency. *Proc. Natl Acad. Sci. USA* **108**, 2361–2365 (2011).
141. Merkel, A. L., Meggers, E. & Ocker, M. PIM1 kinase as a target for cancer therapy. *Expert Opin. Investigat. Drugs* **21**, 425–436 (2012).
142. Yang, J. *et al.* eIF4B phosphorylation by Pim kinases plays a critical role in cellular transformation by Abl oncogenes. *Cancer Res.* **73**, 4898–4908 (2013).
143. Wendel, H. G. *et al.* Dissecting eIF4E action in tumorigenesis. *Genes Dev.* **21**, 3232–3237 (2007).
144. Ostrem, J. M., Peters, U., Sos, M. L., Wells, J. A. & Shokat, K. M. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* **503**, 548–551 (2013).

145. Zimmermann, G. *et al.* Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic KRAS signalling. *Nature* **497**, 638–642 (2013).
References 144 and 145 identify promising new approaches to target oncogenic RAS.
146. Chakrabarty, A. *et al.* Trastuzumab-resistant cells rely on a HER2-PI3K-FoxO-survivin axis and are sensitive to PI3K inhibitors. *Cancer Res.* **73**, 1190–1200 (2013).
147. Donev, I. S. *et al.* Transient PI3K inhibition induces apoptosis and overcomes HGF-mediated resistance to EGFR-TKIs in EGFR mutant lung cancer. *Clin. Cancer Res.* **17**, 2260–2269 (2011).
148. Rexer, B. N. & Arteaga, C. L. Optimal targeting of HER2–PI3K signaling in breast cancer: mechanistic insights and clinical implications. *Cancer Res.* **73**, 3817–3820 (2013).
149. Floris, G. *et al.* A potent combination of the novel PI3K inhibitor, GDC-0941, with imatinib in gastrointestinal stromal tumor xenografts: long-lasting responses after treatment withdrawal. *Clin. Cancer Res.* **19**, 620–630 (2013).
150. Young, C. D. *et al.* Conditional loss of ErbB3 delays mammary gland hyperplasia induced by mutant PIK3CA without affecting mammary tumor latency, gene expression or signaling. *Cancer Res.* **73**, 4075–4085 (2013).
151. Fiskus, W. *et al.* Dual PI3K/AKT/mTOR inhibitor BEZ235 synergistically enhances the activity of JAK2 inhibitor against cultured and primary human myeloproliferative neoplasm cells. *Mol. Cancer Ther.* **12**, 577–588 (2013).
152. Vogt, P. K. & Hart, J. R. PI3K and STAT3: a new alliance. *Cancer Discov.* **1**, 481–486 (2011).
153. Carracedo, A. *et al.* Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J. Clin. Invest.* **118**, 3065–3074 (2008).
154. Kinkade, C. W. *et al.* Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a preclinical mouse model. *J. Clin. Invest.* **118**, 3051–3064 (2008).
155. Zmajkovicova, K. *et al.* MEK1 is required for PTEN membrane recruitment, AKT regulation, and the maintenance of peripheral tolerance. *Mol. Cell* **50**, 43–55 (2013).
156. Posch, C. *et al.* Combined targeting of MEK and PI3K/mTOR effector pathways is necessary to effectively inhibit NRAS mutant melanoma *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **110**, 4015–4020 (2013).
157. Bean, G. R. *et al.* PUMA and BIM are required for oncogene inactivation-induced apoptosis. *Sci. Signal.* **6**, ra20 (2013).
158. Liu, Y. *et al.* Rapamycin induces Bad phosphorylation in association with its resistance to human lung cancer cells. *Mol. Cancer Ther.* **11**, 45–56 (2012).
159. Ellenrieder, V. *et al.* Transforming growth factor β1 treatment leads to an epithelial-mesenchymal transdifferentiation of pancreatic cancer cells requiring extracellular signal-regulated kinase 2 activation. *Cancer Res.* **61**, 4222–4228 (2001).
160. Mulholland, D. J. *et al.* Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. *Cancer Res.* **72**, 1878–1889 (2012).
161. Hsieh, A. C. *et al.* The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature* **485**, 55–61 (2012).
162. Shimizu, T. *et al.* The clinical effect of the dual-targeting strategy involving PI3K/AKT/mTOR and RAS/MEK/ERK pathways in patients with advanced cancer. *Clin. Cancer Res.* **18**, 2316–2325 (2012).
163. Coffee, E. M. *et al.* Concomitant BRAF and PI3K/mTOR blockade is required for effective treatment of *BRAF*^{V600E} colorectal cancer. *Clin. Cancer Res.* **19**, 2688–2698 (2013).
164. Dawson, M. A. *et al.* Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* **478**, 529–533 (2011).
165. Delmore, J. E. *et al.* BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* **146**, 904–917 (2011).
166. Mertz, J. A. *et al.* Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc. Natl Acad. Sci. USA* **108**, 16669–16674 (2011).
167. Zuber, J. *et al.* RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* **478**, 524–528 (2011).
168. Dominguez-Sola, D. & Dalla-Favera, R. Burkitt lymphoma: much more than MYC. *Cancer Cell* **22**, 141–142 (2012).
169. Sander, S. *et al.* Synergy between PI3K signaling and MYC in Burkitt lymphomagenesis. *Cancer Cell* **22**, 167–179 (2012).
This paper establishes an animal model for Burkitt's lymphoma, which requires both MYC and active PI3K.
170. Schmitz, R. *et al.* Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature* **490**, 116–120 (2012).
171. Pourdehnad, M. *et al.* Myc and mTOR converge on a common node in protein synthesis control that confers synthetic lethality in Myc-driven cancers. *Proc. Natl Acad. Sci. USA* **110**, 11988–11993 (2013).
This paper provides evidence that MYC-driven lymphoma is addicted to mTOR activity.
172. Grabher, C., von Boehmer, H. & Look, A. T. Notch 1 activation in the molecular pathogenesis of T-cell acute lymphoblastic leukaemia. *Nature Rev. Cancer* **6**, 347–359 (2006).
173. Guo, D., Teng, Q. & Ji, C. NOTCH and phosphatidylinositide 3-kinase/phosphatase and tensin homolog deleted on chromosome ten/AKT/mammalian target of rapamycin (mTOR) signaling in T-cell development and T-cell acute lymphoblastic leukemia. *Leuk. Lymphoma* **52**, 1200–1210 (2011).
174. Shanware, N. P., Bray, K. & Abraham, R. T. The PI3K, metabolic, and autophagy networks: interactive partners in cellular health and disease. *Annu. Rev. Pharmacol. Toxicol.* **53**, 89–106 (2013).
175. Carayol, N. *et al.* Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells. *Proc. Natl Acad. Sci. USA* **107**, 12469–12474 (2010).
176. Fan, Q. W. *et al.* Akt and autophagy cooperate to promote survival of drug-resistant glioma. *Sci. Signal.* **3**, ra81 (2010).
177. Kao, G. D., Jiang, Z., Fernandes, A. M., Gupta, A. K. & Maity, A. Inhibition of phosphatidylinositol-3-OH kinase/Akt signaling impairs DNA repair in glioblastoma cells following ionizing radiation. *J. Biol. Chem.* **282**, 21206–21212 (2007).
178. Kumar, A., Fernandez-Capetillo, O. & Carrera, A. C. Nuclear phosphoinositide 3-kinase beta controls double-strand break DNA repair. *Proc. Natl Acad. Sci. USA* **107**, 7491–7496 (2010).
179. Ibrahim, Y. H. *et al.* PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. *Cancer Discov.* **2**, 1036–1047 (2012).
180. Juvekar, A. *et al.* Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. *Cancer Discov.* **2**, 1048–1063 (2012).
181. Bassi, C. *et al.* Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. *Science* **341**, 395–399 (2013).
This paper identifies the novel sumoylation and nuclear function of PTEN.
182. Lempiainen, H. & Halazonetis, T. D. Emerging common themes in regulation of PIKKs and PI3Ks. *EMBO J.* **28**, 3067–3073 (2009).
183. Munck, J. M. *et al.* Chemosensitization of cancer cells by KU-0060648, a dual inhibitor of DNA-PK and PI-3K. *Mol. Cancer Ther.* **11**, 1789–1798 (2012).
184. Khalaileh, A. *et al.* Phosphorylation of ribosomal protein S6 attenuates DNA damage and tumor suppression during development of pancreatic cancer. *Cancer Res.* **73**, 1811–1820 (2013).
185. Shen, C. *et al.* Regulation of FANCD2 by the mTOR pathway contributes to the resistance of cancer cells to DNA double strand breaks. *Cancer Res.* **73**, 3393–3401 (2013).
186. Guo, F. *et al.* mTOR regulates DNA damage response through NF-κB-mediated FANCD2 pathway in hematopoietic cells. *Leukemia* **27**, 2040–2046 (2013).
187. Miller, T. W., Balko, J. M. & Arteaga, C. L. Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. *J. Clin. Oncol.* **29**, 4452–4461 (2011).
188. Li, J. *et al.* PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275**, 1943–1947 (1997).
189. Samuels, Y. *et al.* High frequency of mutations of the *PIK3CA* gene in human cancers. *Science* **304**, 554 (2004).
190. Ammirante, M., Luo, J. L., Grivennikov, S., Nedospasov, S. & Karin, M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* **464**, 302–305 (2010).
191. Davids, M. S. & Letai, A. Targeting the B-cell lymphoma/leukemia 2 family in cancer. *J. Clin. Oncol.* **30**, 3127–3135 (2012).
192. Letai, A. *et al.* Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell* **2**, 183–192 (2002).
193. Certo, M. *et al.* Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. *Cancer Cell* **9**, 351–365 (2006).
194. Coloff, J. L. *et al.* Akt-dependent glucose metabolism promotes mcl-1 synthesis to maintain cell survival and resistance to Bcl-2 inhibition. *Cancer Res.* **71**, 5204–5213 (2011).
195. Davids, M. S. *et al.* Decreased mitochondrial apoptotic priming underlies stroma-mediated treatment resistance in chronic lymphocytic leukemia. *Blood* **120**, 3501–3509 (2012).
196. Rahmani, M. *et al.* Dual inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism. *Cancer Res.* **73**, 1340–1351 (2013).
197. Hoellenriegel, J. *et al.* The phosphoinositide 3′-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. *Blood* **118**, 3603–3612 (2011).
This study provides a mechanism for the efficacy of GS-1101 and includes pharmacodynamic data from clinical studies.
198. Motz, G. T. & Coukos, G. Deciphering and reversing tumor immune suppression. *Immunity* **39**, 61–73 (2013).
199. Kalos, M. & June, C. H. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. *Immunity* **39**, 49–60 (2013).
200. Vanneman, M. & Dranoff, G. Combining immunotherapy and targeted therapies in cancer treatment. *Nature Rev. Cancer* **12**, 237–251 (2012).
201. Zitvogel, L., Galluzzi, L., Smyth, M. J. & Kroemer, G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. *Immunity* **39**, 74–88 (2013).
202. Fruman, D. A. & Bismuth, G. Fine tuning the immune response with PI3K. *Immunol. Rev.* **228**, 253–272 (2009).
203. Okkenhaug, K. Signaling by the phosphoinositide 3-kinase family in immune cells. *Annu. Rev. Immunol.* **31**, 675–704 (2013).
204. Jiang, Q. *et al.* mTOR kinase inhibitor AZD8055 enhances the immunotherapeutic activity of an agonist CD40 antibody in cancer treatment. *Cancer Res.* **71**, 4074–4084 (2011).
205. Li, Q. *et al.* A central role for mTOR kinase in homeostatic proliferation induced CD8$^+$ T cell memory and tumor immunity. *Immunity* **34**, 541–553 (2011).
206. Marshall, N. A. *et al.* Immunotherapy with PI3K inhibitor and Toll-like receptor agonist induces IFN-γ$^+$IL-17$^+$ polyfunctional T cells that mediate rejection of murine tumors. *Cancer Res.* **72**, 581–591 (2012).
This paper shows that PI3K inhibitors can enhance the adjuvant activity of Toll-like receptor agonists to improve dendritic cell-based tumour vaccines in mice.
207. Yao, E. *et al.* Suppression of HER2/HER3-mediated growth of breast cancer cells with combinations of GDC-0941 PI3K inhibitor, trastuzumab, and pertuzumab. *Clin. Cancer Res.* **15**, 4147–4156 (2009).
208. Mao, M. *et al.* Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. *Clin. Cancer Res.* **19**, 657–667 (2013).
209. Paraiso, K. H. *et al.* PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. *Cancer Res.* **71**, 2750–2760 (2011).
210. Nardella, C., Lunardi, A., Patnaik, A., Cantley, L. C. & Pandolfi, P. P. The APL paradigm and the "co-clinical trial" project. *Cancer Discov.* **1**, 108–116 (2011).
211. Suire, S. *et al.* Gβγs and the Ras binding domain of p110γ are both important regulators of PI(3)Kγ signalling in neutrophils. *Nature Cell Biol.* **8**, 1303–1309 (2006).
212. Delgado, P. *et al.* Essential function for the GTPase TC21 in homeostatic antigen receptor signaling. *Nature Immunol.* **10**, 880–888 (2009).

213. Rodriguez-Viciana, P., Sabatier, C. & McCormick, F. Signaling specificity by Ras family GTPases is determined by the full spectrum of effectors they regulate. *Mol. Cell. Biol.* **24**, 4943–4954 (2004).
214. Dbouk, H. A. *et al.* G protein-coupled receptor-mediated activation of p110β by Gβγ is required for cellular transformation and invasiveness. *Sci. Signal.* **5**, ra89 (2012).
215. Durand, C. A. *et al.* Phosphoinositide 3-kinase p110δ regulates natural antibody production, marginal zone and B-1 B cell function, and autoantibody responses. *J. Immunol.* **183**, 5673–5684 (2009).
216. Reif, K. *et al.* Cutting edge: differential roles for phosphoinositide 3-kinases, 110γ and p110δ, in lymphocyte chemotaxis and homing. *J. Immunol.* **173**, 2236–2240 (2004).
217. Puri, K. D. & Gold, M. R. Selective inhibitors of phosphoinositide 3-kinase delta: modulators of B-cell function with potential for treating autoimmune inflammatory diseases and B-cell malignancies. *Frontiers Immunol.* **3**, 256 (2012).
218. Ghosh, B. *et al.* Nontoxic chemical interdiction of the epithelial-to-mesenchymal transition by targeting cap-dependent translation. *ACS Chem. Biol.* **4**, 367–377 (2009).
219. Knauf, U., Tschopp, C. & Gram, H. Negative regulation of protein translation by mitogen-activated protein kinase-interacting kinases 1 and 2. *Mol. Cell. Biol.* **21**, 5500–5511 (2001).
220. Lim, S. *et al.* Targeting of the MNK-eIF4E axis in blast crisis chronic myeloid leukemia inhibits leukemia stem cell function. *Proc. Natl Acad. Sci. USA* **110**, E2298–E2307 (2013).
221. Konicek, B. W. *et al.* Therapeutic inhibition of MAP kinase interacting kinase blocks eukaryotic initiation factor 4E phosphorylation and suppresses outgrowth of experimental lung metastases. *Cancer Res.* **71**, 1849–1857 (2011).
222. Lin, Y. W. *et al.* A small molecule inhibitor of Pim protein kinases blocks the growth of precursor T-cell lymphoblastic leukemia/lymphoma. *Blood* **115**, 824–833 (2010).
223. Blanco-Aparicio, C. *et al.* Pim 1 kinase inhibitor ETP-45299 suppresses cellular proliferation and synergizes with PI3K inhibition. *Cancer Lett.* **300**, 145–153 (2011).
224. Chen, L. S., Redkar, S., Bearss, D., Wierda, W. G. & Gandhi, V. Pim kinase inhibitor, SGI-1776, induces apoptosis in chronic lymphocytic leukemia cells. *Blood* **114**, 4150–4157 (2009).
225. Song, J. H. & Kraft, A. S. Pim kinase inhibitors sensitize prostate cancer cells to apoptosis triggered by Bcl-2 family inhibitor ABT-737. *Cancer Res.* **72**, 294–303 (2012).
226. Pogacic, V. *et al.* Structural analysis identifies imidazo[1,2-b]pyridazines as PIM kinase inhibitors with *in vitro* antileukemic activity. *Cancer Res.* **67**, 6916–6924 (2007).
227. Rommel, C. et al. Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt. *Science* **286**, 1738–1741 (1999).
228. Zimmermann, S. & Moelling, K. Phosphorylation and regulation of Raf by Akt (protein kinase B). *Science* **286**, 1741–1744 (1999).

Acknowledgements
The authors thank J. Oliner for critical review of the manuscript, and B. Vanhaesebroeck for sharing unpublished results. Research on PI3K and mTOR in David Fruman's laboratory is supported by US National Institutes of Health (NIH) grants CA158383 and AI099656, and the Cancer Center Support Grant P30CA62203 to University of California, Irvine (UC Irvine).

Competing interests statement
The authors declare competing interests: see Web version for details.

DATABASES
ClinicalTrials.gov website: http://www.clinicaltrials.gov

SUPPLEMENTARY INFORMATION
See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF