EXPLOITING THE PI3K/AKT PATHWAY FOR CANCER DRUG DISCOVERY

Bryan T. Hennessy, Debra L. Smith, Prahlad T. Ram, Yiling Lu and Gordon B. Mills

Abstract | Evolving studies with several different targeted therapeutic agents are demonstrating that patients with genomic alterations of the target, including amplification, translocation and mutation, are more likely to respond to the therapy. Recent studies indicate that numerous components of the phosphatidylinositol-3-kinase (PI3K)/AKT pathway are targeted by amplification, mutation and translocation more frequently than any other pathway in cancer patients, with resultant activation of the pathway. This warrants exploiting the PI3K/AKT pathway for cancer drug discovery.

The phosphatidylinositol-3-kinase (PI3K) signalling pathway is crucial to many aspects of cell growth and survival. It is targeted by genomic aberrations including mutation, amplification and rearrangement more frequently than any other pathway in human cancer, with the possible exception of the p53 and retinoblastoma (Rb) pathways, although these pathways crosstalk at multiple levels and constitute a signalling network implicated in tumour initiation and progression. In addition, the PI3K pathway is stimulated as a physiological consequence of many growth factors and regulators. Whatever the mechanism, activation of the PI3K pathway results in a disturbance of control of cell growth and survival, which contributes to a competitive growth advantage, metastatic competence and, frequently, therapy resistance. This pathway is therefore an attractive target for the development of novel anticancer agents.

Opportunities and challenges

In contrast to p53 and other tumour-suppressor pathways, the PI3K pathway is activated in cancer, making this an optimal target for therapy as it is easier to inhibit activation events than to replace lost tumour-suppressor function. More than 20 companies and academic centres have declared active programmes in this area (TABLE 1). Despite major interest and widespread screening, no drugs developed to specifically target the pathway have progressed to cancer clinical trials, although several are likely to enter trials in the next year. A number of drugs in clinical use or preclinical evaluation originally developed for other purposes or identified in non-PI3K pathway screens have been demonstrated to directly or indirectly target PI3K signalling. These include mammalian target of rapamycin (mTOR) inhibitors of the 'rapalog' family of rapamycin analogues, ether lipids (such as perifosine and miltefosine), and inhibitors of epidermal growth factor receptor (EGFR), HER2/*neu*, c-Kit, platelet-derived growth factor receptor (PDGFR) and BCR–ABL. However, with the exception of mTOR inhibitors, which seem to solely target the PI3K pathway, whether functional outcomes of these drugs relate to inhibition of the PI3K pathway or to other effects is unknown. Because of the frequency of PI3K mutations and signalling abnormalities in neoplasia and their multiple downstream effects, it is expected that an expanding repertoire of drugs targeting the PI3K pathway will be rapidly developed, evaluated and incorporated into the management of different cancers. As the PI3K pathway is important for many normal cellular functions and, in particular, signalling by insulin, the main limiting factor to implementing drugs that inhibit the pathway will probably

Department of Molecular Therapeutics, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston TX 77030, USA. Correspondence to G.B.M. e-mail: gmills@mdanderson.org
doi:10.1038/nrd1902

Table 1 | **Current drugs in development that target the PI3K or related pathways**

Effect on pathway	Target	Examples	Company/centre	Status
Direct	PI3K	Ly294002	Lilly	Poor pharmacology
		Wortmannin analogues	PROLX	Preclinical
		PX-866	Lilly	Preclinical leads
		SF1124	Semafore	Preclinical leads
		PEG Wortmannin	Echelon	Preclinical
			Wyeth	Preclinical
			Baxter	Preclinical
			ICOS	Preclinical
	P110δ		Piramed	
	P110α		Piramed	
	Pan-inhibitor		Cerylid	
		KN309	Cerylid/Kinacia	
	PDK1		Berlex Lilly ICOS Vertex	All are preclinical
	ILK		QLT	Preclinical
	AKT kinase domain		QLT	Preclinical
			Abbott	
			Novartis	
			Lilly	
			Vertex	
			Roche	
			Celgene	
			Novartis	
			Kinacia/Cerylid	
		PX316	BioImage	Screening
			PROLX	Preclinical
	PH domain	Miltefosine	Zentaris	Approved in Europe for breast cancer
		Perifosine	Keryx	In clinical trial for leishmaniasis
				Phase II
			NIH	Preclinical
			Schering	
			Merck	
			Celgene	
	mTOR	Rapamycin	Wyeth	
		CCI779	Wyeth/NCI/CTEP	Approved
		Rad 001	Novartis	Phase II
		AP23573	Ariad	Phase II
		AP23841	Ariad	Phase II
		AP23573	Ariad	Preclinical
	$P70^{S6}$ kinase		Lilly	
	Forkhead translocation	Calmodulin inhibitors	Harvard Bioimage	Clinical trials Preclinical
Indirect	Growth factor receptors	EGFR HER2 Insulin Integrins	Multiple	Preclinical to approved
	Intracellular kinases	Src Abl	Multiple	

EGFR, epidermal growth factor receptor; ILK1, integrin-linked kinase; mTOR, mammalian target of rapamycin; NIH, National Institutes of Health; PDK1, phosphoinositide-dependent kinase 1; PI3K, phosphatidylinositol-3-kinase.

be the identification of targets and drugs with a sufficient therapeutic index to warrant clinical implementation. Whether these drugs will demonstrate antitumour activity alone or in combination with other agents is unknown. In particular, as the PI3K pathway is a crucial regulator of survival during cellular stress, and given that tumours frequently exist in intrinsically stressful environments with limited nutrient and oxygen supply and low pH, inhibition of the PI3K pathway is likely to find optimal efficacy in combination approaches to induce cell stress, including combination with other signal-transduction inhibitors and with chemotherapy or radiation therapy.

As PI3K pathway inhibitors are integrated into clinical practice, it will be crucial to develop methods to identify those patients with tumours 'driven' by molecular abnormalities that can be exploited by these

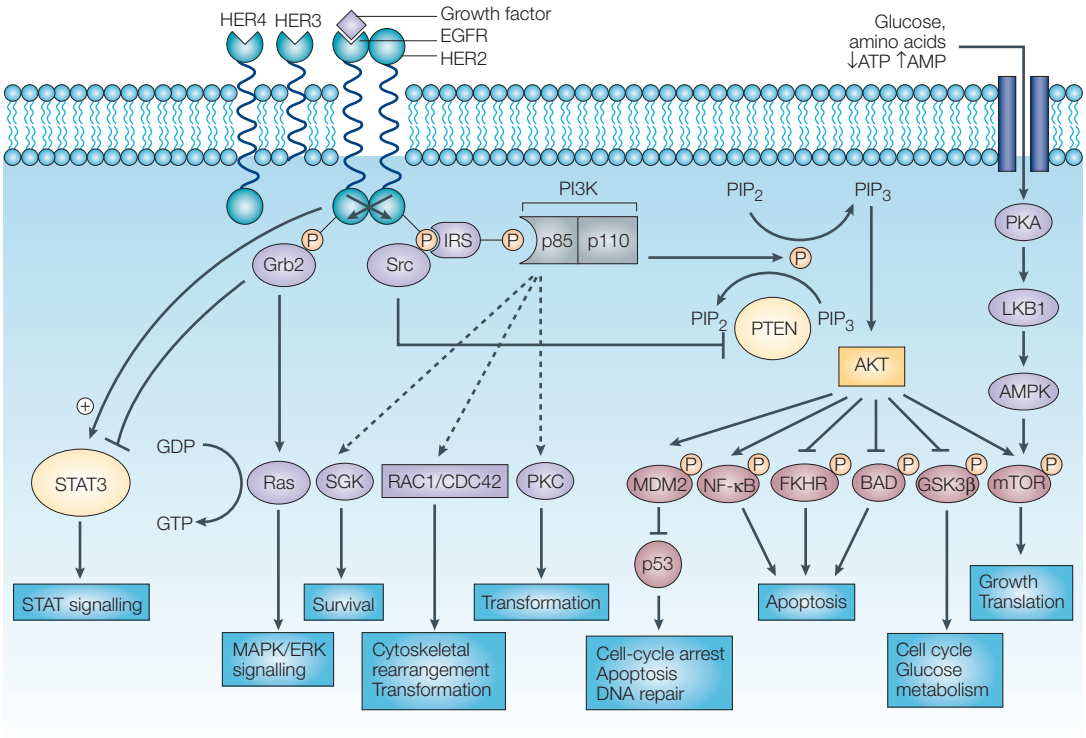


Figure 1 | **Schematic of signalling through the phosphatidylinositol-3-kinase (PI3K)/AKT pathway.** The PI3K/AKT and related pathways are important in internalizing the effects of external growth factors and of membrane tyrosine kinases. Activation of membrane kinases including epidermal growth factor receptor (EGFR) by external growth factors initiates receptor dimerization and subsequent events to activate these intracellular pathways. AKT is activated downstream of PI3K and has multiple targets. AKT and the cellular energy sensors LKB1 (STK11) and AMP-activated protein kinase (AMPK) exert opposing effects on mammalian target of rapamycin (mTOR), which is activated by AKT. ERK, extracellular signal regulated kinase; FKHR, forkhead; GDP, guanosine diphosphate; IRS, insulin receptor substrate; GSK3, glycogen synthase kinase 3; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PIP_2, phosphatidylinositol-3,4-diphosphate; PIP_3, phosphatidylinositol-3,4,5-triphosphate; PKC, protein kinase C; STAT, signal transducer and activator of transcription.

inhibitors. As it is not presently clear whether patients with aberrations of particular isoforms or molecules in the pathway will require treatment with drugs targeting particular points in the pathway, it will be important to co-develop molecular markers and targeted therapeutics. This approach will maximize the efficacy and cost effectiveness of these new therapies and minimize needless patient exposure. Further, an ability to pre-select patients who are likely to respond, and to identify patients not responding at an early point during treatment and triage them to alternative therapies, will greatly increase the likelihood of demonstrating efficacy and decrease the size, cost and duration of clinical trials while concurrently protecting patent life, an approach favoured by drug companies and recently the FDA. Once a targeted therapeutic is approved for use in a selected population of patients, post-marketing studies can be used to identify the spectrum of diseases and combination therapies most effective. This approach was highly successful with imatinib mesylate (Gleevec; Novartis), which was developed for treatment of chronic myeloid leukaemia (CML) based on CML being almost exclusively defined by the *BCR–ABL* fusion oncogene. Imatinib was subsequently found to be highly effective in targeting genetic aberrations in other diseases, such as mutational activation of c-Kit in gastrointestinal stromal tumours (GIST) or the PDGFR in dermatofibrosarcoma protuberans and hypereosinophilic syndrome.

The PI3K/AKT pathway: overview

PI3Ks. The PI3K family constitutes a large family of lipid and serine/threonine kinases, which includes a number of phosphatidylinositol kinases, as well as the related DNA-dependent protein, ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) kinases[1–4]. Class 1A PI3Ks are composed of heterodimers of an inhibitory adaptor/regulatory (p85) and a catalytic (p110) subunit. p85 binds and integrates signals from various cellular proteins, including transmembrane tyrosine kinase-linked receptors and intracellular proteins such as protein kinase C (PKC), SHP1, Rac, Rho, hormonal receptors, mutated Ras and Src, providing an integration point for activation of p110 and downstream molecules (FIG. 1). The SH2 domain of p85 has two major divergent functional activities: activation of small G-proteins and relief of *trans*-inhibition of p110.

Upon activation, PI3Ks phosphorylate phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2; FIG. 2)

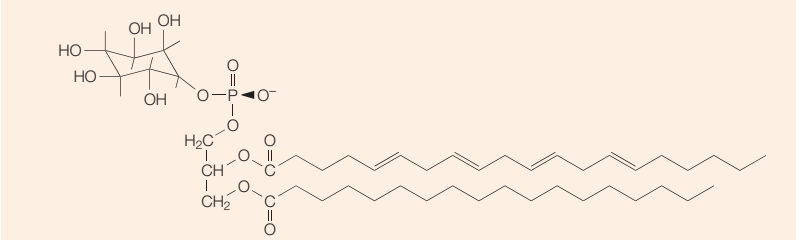


Figure 2 | **The structure of phosphatidylinositol.** The free hydroxyls are at positions 2–6 of the inositol head.

to produce PtdIns(3,4,5)P_3, a second messenger that binds a subset of pleckstrin-homology (PH), FYVE, Phox (PX), C1, C2 and other lipid-binding domains in downstream targets to recruit them to the activation nidus at the membrane. The PH domain is the predominant domain involved in this interaction although only a subset of PH domains bind to PtdIns(3,4,5)P_3, providing one level of specificity to the interaction. Genetic screens in model organisms have identified AKT as the primary downstream mediator of the effects of PI3K; however, the presence of a large number of proteins with FYVE, PH and other lipid-binding domains that interact with PtdIns(3,4,5)P_3 suggest additional crucial targets. PtdIns(3,4,5)P_3 is subsequently metabolized by SHIP-1 and -2 to generate PtdIns(3,4)P_2, which regulates a separate subset of PH domains and thus downstream signalling molecules[1–4]. PTEN dephosphorylates the 3′OH group phosphorylated by PI3K, acting as the 'yin' tumour suppressor to the 'yang' oncogene, PI3K.

There are three known isoforms of Class IA p110 (p110α/p110β/p110δ), which contain an amino-terminal p85/p55-interacting region, a domain that binds to Ras, a 'PIK domain' homologous to other phosphoinositide kinases, and a carboxy-terminal catalytic domain. There are seven known p85/p55 subunits generated by alternative splicing of three genes (p85α/p85β/p55γ); all can bind p110α/β/δ[1]. Biochemical and gene knockout studies suggest that the different isoforms of p110 and p85 preferentially mediate specific signalling processes, with, however, a degree of redundancy. TABLE 2 lists some differential functions associated with p110 isoforms[7–21]. Only p110α and p85α have been found to be mutated and p85α to be translocated in tumours (see below). Further, insulin and some growth factors preferentially signal through p110β. This suggests that it might be necessary to develop isoform-specific inhibitors to decrease toxicity or pan inhibitors to increase efficacy. The balance between efficacy and toxicity (therapeutic index) can only be defined empirically using chemical or functional genomics.

Class 1B PI3Ks consist of p110γ and a regulatory subunit, p101, and are activated directly by G-protein-coupled receptors and indirectly by other receptors. All class I PI3Ks possess intrinsic protein kinase activity; p110 autophosphorylation and phosphorylation of p85 downregulate activity of the complex. Two chemical inhibitors have been used to probe the function of PI3K: wortmannin, a fungal metabolite that irreversibly inhibits p110 by reacting covalently with the catalytic site[5], and the flavenoid derivative LY294002, a reversible inhibitor which, like wortmannin, inhibits all class I PI3Ks. However, both inhibitors have off-target activity. As a result, the effects of these inhibitors might not solely be a consequence of PI3K inhibition. Many inhibitors with greater selectivity are under development, providing higher-quality chemical probes with which to study the function of the PI3K pathway and to explore the potential therapeutic indices of novel inhibitors.

Class II PI3Ks are monomeric, lack adapter subunits and preferentially use PtdIns and PtdIns(4)P as substrates[6]. Three mammalian class II isoforms have been identified: the ubiquitously expressed PI3K-C2α and PI3K-C2β, and liver-specific PI3K-C2γ. Class III PI3Ks are heterodimeric enzymes consisting of adaptor (p150) and catalytic (Vps34, 100 kDa) subunits; the latter produce only PtdIns(3)P, are thought to be central to vesicle trafficking and lack a Ras-binding domain. Intriguingly, class III PI3K has been implicated in regulation of autophagy, a phylogenetically conserved process that enables survival under conditions of cell stress.

AKT (protein kinase B). AKT, the human homologue of the viral oncogene *v-akt*, is related to protein kinases A (PKA) and C (PKC) in humans[22–24]. The three known AKT isoforms are derived from distinct genes (AKT1/PKBα, AKT2/PKBβ and AKT3/PKBγ). The PH domain in the N-terminal region of AKT interacts with 3′-phosphoinositides, contributing to recruitment of AKT to the plasma membrane. However, our recent studies suggest that the PH domain is not sufficient for recruitment to the membrane and that AKT interaction partners also have a crucial role. Recruitment to the membrane results in a conformational change that exposes two crucial amino acids that are phosphorylated and necessary for activation: one in the kinase domain (threonine 308 in AKT1) is phosphorylated by constitutively active phosphoinositide-dependent kinase 1 (PDK1), stabilizing the activation loop, whereas phosphorylation of the other in the hydrophobic C-terminal domain (serine 473 in AKT1) by PDK2 is necessary for full activation[25,26]. Several different potential PDK2s have been identified, including the mTOR rictor complex (but not the mTOR raptor complex inhibited by rapamycin and its analogues), integrin-linked kinase (ILK), PKCβII and even AKT itself, thereby allowing the pathway potential for feedback control[27–29]. How these potential PDK2s interact to regulate AKT is not yet understood. The relative roles of AKT signalling at the membrane, the cytosol and the nucleus also remain to be determined.

Isoforms of AKT have been implicated in specific functions in cancer (AKT2 in motility/invasion and AKT3 in hormone independence)[30]. In knockout mice, elimination of different isoforms leads to different developmental defects and alterations in

Table 2 | **Differential functions associated with p110 isoforms of PI3K**

Isoform	Function
p110α	Deletion associated with defective mouse embryonic proliferation and death at 10 days[7]
	Mutated/amplified in human cancer. Constitutively active cardiac-specific isoform leads to cardiomyopathy in mice[6]
p110β	Insulin signalling[8]
	Lysophosphatidic acid signalling[8]
	Cancer-cell motility[14]
	Vascular smooth-muscle cell chemotaxis[16]
	Phagocytosis by macrophages[18]
	Deletion associated with profound mouse embryonic proliferation reduction and death at 3–7 days
	Platelet thrombus formation[21]
p110δ	B-cell development and function and B- and T-cell antigen receptor signalling[9]
	IL-4 receptor signalling[10]
	Artery smooth-muscle tone and hypertension[11]
	Neutrophil migration and primed neutrophil burst (phase II)[12]
	Cell proliferation in acute myeloid leukaemia[13]
	Expressed in endothelial cells[15]
	In mice, expressed predominantly in leukocytes[19]
	Mouse kinase inactive knockin mutant impairs immunity[9]
p110γ	Primed neutrophil burst (phase I)[12]
	Pathological responses of pancreatic acinar cell[17]
	Speculated involvement in pancreatitis
	Embryonic mice knockout models viable with increased myocardial contractility
	Might play a detrimental role in heart failure[20]

IL-4, interleukin-4; PI3K, phosphatidylinositol-3-kinase.

insulin sensitivity[31–33]. AKT2 is genomically amplified in pancreatic, breast and ovarian tumours, and AKT3 is overexpressed by an unknown mechanism in hormone-insensitive breast and prostate cancers[34]. Aberrations in AKT1 are much less frequent. AKT2 and AKT3 might therefore fulfil different functions, resulting in selective pressure for aberrations during tumorigenesis. As with PI3K, isoform-selective inhibitors might be needed for optimal efficacy with acceptable toxicity.

PI3K/AKT-regulated downstream cellular events

PI3K in cell survival and apoptosis inhibition. Previous reviews have provided depth and focus on the role of the PI3K/AKT pathway in cell proliferation and survival[35,36]. We will therefore review only a few salient points with applicability to drug development. AKT signalling inactivates several proapoptotic factors (FIG. 1)[37,38]. These include BAD, procaspase-9 and Forkhead (FKHR) transcription factors. AKT also activates transcription factors that upregulate anti-apoptotic genes, including cyclic-AMP response element-binding protein (CREB), and activates IκB kinase (IKK) to phosphorylate IκB (inhibitor of NF-κB), leading to its proteasomal degradation and NF-κB nuclear localization. AKT can inactivate p53 through mdm2, contributing to centrosome hyperamplification and chromosome instability in cancer[39–41]. AKT phosphorylation of many effectors regulates their localization, and thereby activity, by generating binding sites for 14-3-3 proteins, which are important in regulating cellular location and degradation of various molecules[42,43].

PI3K in cell growth, metabolism, translation and proliferation. The PI3K pathway bifurcates at many points, resulting in diverse functional outcomes. The panoply of functional responses probably represents the effects of the spectrum, level and duration of activation of particular components of the PI3K pathway. However, different nodes in the pathway have been implicated as playing a dominant role in particular outcomes (FIG. 1). AKT-mediated activation of mTOR is important in stimulating cell proliferation[44]. mTOR regulates translation in response to nutrients/growth factors by phosphorylating components of the protein synthesis machinery, including the ribosomal protein S6 kinases ($p70^{S6K}$) and 4E-binding protein (4E-BP), the latter resulting in release of the translation initiation factor eIF4E, which is known to have transforming and anti-apoptotic activities *in vitro*[44–47]. Intriguingly, rapamycin and its analogues selectively inhibit the effects of mTOR on $p70^{S6K}$ while sparing 4E-BP phosphorylation. The tuberous sclerosis complex-1 (TSC1):TSC2 complex opposes these effects by inhibiting $p70^{S6K}$ and activating 4E-BP1 to sequester eIF4E[48–51]. However, AKT also phosphorylates and inhibits TSC2, and so this pathway is controlled by complicated interactions and feedback loops. The TSC/Rheb/mTOR/S6K cascade also regulates insulin receptor substrate (IRS) 1/2 and PDGFR, which potentially comprises an additional important feedback loop[52,53]. Indeed, mTOR inhibition by rapamycin or rapamycin analogues can activate upstream proteins including AKT, which is probably a result of loss of feedback inhibition[54,55]. Activated AKT, in part via eIF4E, can then attenuate growth inhibition associated with rapamycin and its analogues. Targeting the PI3K pathway at multiple sites might therefore be required to interrupt feedback loops to achieve optimal outcomes. Indeed, combining rapamycin with LY294002 or with inhibitors of cell-surface tyrosine kinases enhances growth inhibitory activity *in vitro*, warranting the evaluation of combinatorial therapy in animal models and, eventually, patients.

In addition, the PI3K/AKT pathway interacts with molecular mechanisms controlling cellular energy control and glucose metabolism. LKB1 (STK11)-mediated activation of AMP-activated protein kinase (AMPK), an evolutionarily conserved sensor of the cellular ATP/ADP ratio, leads to inhibition of mTOR through TSC2 in response to energy depletion, thereby allowing energy conservation[56,57]. Germline

HAMARTOMAS
Abnormal growth of mature normal cells and tissues in an organ composed of identical elements.

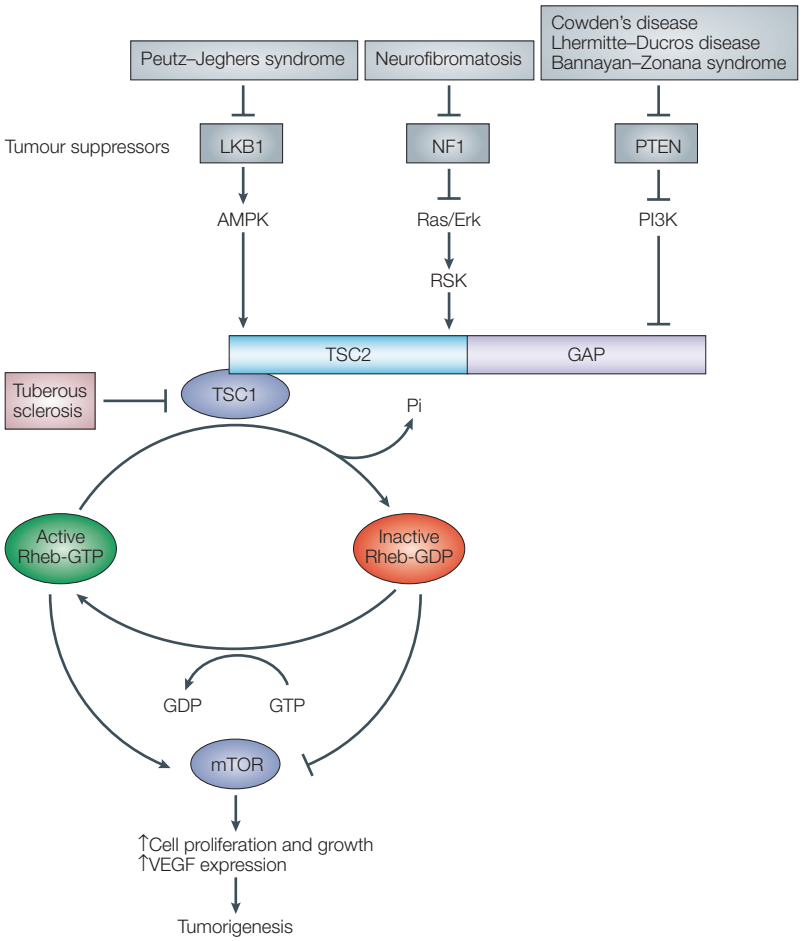


Figure 3 | **Clinical syndromes associated with altered PI3K pathway signalling resulting from mutations in four tumour-suppressor genes.** The tumour-suppressor genes LKB1 (STK11), PTEN, NF1 (neurofibromatosis 1), and tuberous sclerosis complex (TSC) 1/2 all exert interacting inhibitory effects on signalling through the phosphatidylinositol-3-kinase (PI3K)/AKT pathway through the TSC complex that converts Rheb-GTP, which activates mTOR, to inactive Rheb-GDP. When each is 'knocked out' by germline mutation, the result is a clinically manifest syndrome characterized by an increased risk of malignancy. ERK, extracellular signal regulated kinase; GAP, GTPase activating protein; mTOR, mammalian target of rapamycin; Rheb, atypical member of the Ras family with an unusually low intrinsic GTPase activity; VEGF, vascular endothelial growth factor.

mutations in genes encoding PTEN, TSC2 and LKB1 all result in similar clinical syndromes that are characterized by the presence of HAMARTOMAS at different sites and an increased risk of specific malignancies, providing evidence that deregulation of the PI3K/AKT pathway by inactivation of these crucial genes causes loss of cellular growth regulation (FIG. 3)[58]. AKT phosphorylates both cyclin-dependent kinase (CDK) inhibitors $p21^{CIP1/WAF1}$ and $p27^{KIP1}$. This results in their exclusion from the nucleus and subsequent cytoplasmic sequestration/degradation[59–61]. This increases cellular proliferation due to decreased inhibition of cyclins and also likely due to novel cytosolic functions of the CDK inhibitors.

AKT, directly or indirectly, phosphorylates and inhibits glycogen synthase kinase-3 (GSK3), phosphodiesterase-3B, protein phosphatase 2A and possibly Raf1, creating a complex intracellular network. GSK3 inhibition prevents phosphorylation of β-catenin, thereby impeding its degradation and resulting in its translocation to the nucleus to stimulate transcription of target genes including *c-JUN* and the homeobox gene *CDX1*. In addition, cyclin D1 phosphorylation by GSK3 results in its destabilization[62].

PI3K signalling also controls angiogenesis, growth, proliferation, senescence and other processes through mechanisms including vascular endothelial growth factor (VEGF) transcriptional activation and induced hypoxia inducible factor-1α (HIF1α) expression[63–67]. The von Hippel Lindau (vHL) tumour-suppressor protein, through its oxygen-dependent polyubiquitylation of HIF1α leading to HIF1α degradation, has a central role in the mammalian oxygen-sensing pathway by opposing the effects of the PI3K pathway.

Role of the PI3K/AKT pathway in therapy resistance. Inhibition of components of the PI3K pathway can synergize with, or overcome resistance to, chemotherapy, radiation therapy, hormone therapy and targeted agents in cancer[68–73]. Potential mechanisms for this synergy include potentiation of apoptosis. However, most of these studies have been performed with relatively nonspecific compounds and could reflect inhibition of PI3K-like kinases activated by DNA damage/radiation (for example, DNA-activated protein kinase (DNA-PK), ATM and ATR) by drugs such as LY294002 or wortmannin. In breast cancer, there are important interactions between the genomic and non-genomic effects of the oestrogen receptor and cytoplasmic/membrane kinases, including members of the PI3K pathway that play a role in anti-oestrogen resistance[74,75]. PTEN activity contributes to the efficacy of trastuzumab (Herceptin; Genentech) and radiation therapy[76]. The activation state of the PI3K pathway therefore contributes to tumour resistance to targeted therapeutics as well as to chemo/radiation therapy.

PI3K pathway genetic aberrations in cancer

Abnormalities in the PI3K pathway are common in cancer and have a role in neoplastic transformation (TABLE 3)[77]. PI3K itself is a frequent target of mutational activation[78–80]. The most frequent genetic aberrations in breast cancer are somatic missense mutations in the gene encoding p110α (*PIK3CA*); these mutations occur most frequently in HER2-amplified and hormone-receptor-positive breast cancers. Amplification or mutation of the *PIK3CA* gene also commonly occur in bowel cancer, ovarian cancer (mutations in endometrioid and clear-cell subtypes and amplification in serous tumours), head and neck and cervical squamous cancers, gastric and lung cancers, anaplastic oligodendrogliomas, anaplastic astrocytomas, glioblastoma multiforme and medulloblastomas[81–86]. Although p85α mutations and translocations are rare, they serve to emphasize the importance of the pathway[87]. AKT and PTEN are also targets of frequent genomic and epigenetic changes

Table 3 | **Abnormalities in the PI3K/AKT signalling pathway in cancer**

Molecule	Alteration in tumours	Frequency	Tumour lineage
PTEN	Mutations (somatic)	>50%	Glioma, melanoma, prostate cancer Endometrial cancer, endometrioid ovarian cancer Variable in sporadic breast cancers (2–30%)
PTEN	Decreased expression Methylation Loss of heterozygosity	>50%	Breast, melanoma, prostate Microsatellite instability-high colorectal cancer Endometrial cancer Leukaemia
PTEN	Germline mutations	80% of Cowden's disease	High risk of breast, thyroid and endometrial carcinomas
p85	Activating mutations	Rare	Ovary, colon, glioma, lymphoma cell line (CO)
p85	Fusion	Very rare	Lymphoma
p55γ (isoform of p85)	Deletion mutations	Unknown	Lung cancer cell line (HCC15)
PIK3CA	Amplification	Up to 50% Rare	Ovary, cervix, lung Breast (BRCA1 associated?)
PIK3CA	Activating mutation	>50% >25%	Bowel Breast
AKT1	Amplification	Low	Gastric
AKT2	Amplification	Low	Ovary (12–25%) Pancreas (20%), breast (rare)
AKT2	Mutation	Low	Colorectal
AKT3	Overexpression	Low	Hormone-resistant prostate and breast cancer
PDK1	Mutation	Low	Colorectal
$p70^{S6}$ kinase	Amplification	30%	Breast
TSC1/2	Mutation	>50%	Tuberous sclerosis
Forkhead family	Translocations	>50% Low	Alveolar rhabdomyosarcoma Acute leukaemia
TCL1	Rearrangement	Unclear	T-cell leukaemia Chronic lymphocytic leukaemia

EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol-3-kinase; *PIK3CA*, gene encoding the p110α PI3K subunit; PDK1, phosphoinositide-dependent kinase-1; TCL1, T-cell leukaemia 1; TSC, tuberous sclerosis complex.

in human cancers (TABLE 3)[88–98]. Recently, systematic, large-scale gene sequencing of tyrosine kinases and phosphatases by three academic groups (see Sanger Centre Catalogue of Somatic Mutations in Cancer in Further information) has proven informative[99–102]. Although no frequent novel mutation was discovered in the PI3K or other kinase pathways, previously undescribed infrequent mutations were detected — for example, in three genes encoding PI3K/AKT pathway components (PDK1, AKT2 and p21-activated kinase-4 (PAK4)) in colorectal cancer. The avian p110 catalytic subunit orthologue and mutant p85 are directly transforming, and, in specific models, AKT isoforms demonstrate transforming activity.

Abnormalities also commonly occur in related molecules and signalling pathways in cancer (TABLE 4)[103–117]. In many cases, the oncogenic effects of these abnormalities are mediated at least partly through PI3K/AKT signalling. Ras mutations, which activate PI3K, are common in pancreatic cancer[103–106]. Modulation of the PI3K/AKT pathway is required for HER2-mediated tumorigenesis in animal models and for responses to trastuzumab[108–112]. The PI3K/AKT pathway is also required for the oncogenic effects of EGFR. Increased PI3K activity is associated with transformation by SRC, polyoma middle T antigen, ABL and Ros.

Drug development targeting PI3K

Given the importance of the PI3K pathway, new candidates join the panoply of drugs under evaluation frequently. TABLE 1 shows a partial list of drugs in development to exploit the PI3K and related signalling pathways. The drugs fall into several classes. Many of the candidates were first explored to target other molecules or for uses other than cancer therapy. A number of the candidates target all isoforms of a particular protein in the PI3K pathway. A smaller number of isoform-selective inhibitors have been identified. The inhibitors have been derived from large-scale screens as well as from rational drug design using known crystal structures. Further, multiple different components of the pathway are being explored as potential targets. The eventual success or failure of these drugs will depend on their therapeutic index as well as the ability to identify patients likely to respond to particular therapeutics. The relative role of

each potential target in the PI3K pathway will need to be determined with high-quality drugs and might only be applicable in particular populations of patients. For example, a patient with a downstream-activating mutation is unlikely to respond to a drug targeting an upstream component of the pathway. Further, crucial proximal nodes such as AKT might not be druggable due to toxicity, whereas downstream targets such as mTOR could fail to demonstrate sufficient efficacy. In the absence of appropriate chemical probes, it is impossible to predict the outcomes of pan-inhibitors compared with isoform-specific inhibitors, particularly in terms of therapeutic index. Although functional genomics approaches, such as small interfering RNA (siRNA), might provide clues about the effect of inhibitors of specific isoforms of targets, it is likely that target knockdown will show different functional outcomes than an inhibited molecule that could act in a dominant negative fashion or exhibit gain-of-function activities. Based on the complexity of PI3K signalling, crosstalk with multiple pathways, and the presence of potent feedback loops, combined with its importance in multiple physiological functions, combinations of targeted therapeutics might be required for efficacy and safety.

PI3K inhibitors. Wortmannin and LY294002 have antitumour activity *in vitro* and *in vivo*, and sensitize tumour cells to other targeted therapeutics, chemotherapy and radiation[118–123]. Stable water-soluble conjugates of wortmannin are being developed to improve its pharmacological characteristics. LY294002 has a very short half-life and is insoluble in aqueous solutions. Novel LY294002 prodrugs are in development. A number of companies and academic sites have developed additional pan and isoform-selective PI3K inhibitors[124–127]. Although none of these inhibitors have been evaluated in human trials, preclinical data and animal models indicate that the new-generation inhibitors have much better pharmacological characteristics: greater water solubility, less protein binding, better pharmacodynamics, as well as improved PI3K selectivity. A number of isoform-specific inhibitors are under evaluation but as yet have not demonstrated superior activity or less toxicity than LY294002 or wortmannin. With the advent of small interfering RNA (siRNA) technologies and recent demonstration of *in vivo* activity in several tumour models, it might be possible to selectively target the p110 or p85 isoforms. Indeed, the selectivity of siRNA is such that it could potentially target primarily the mutant allele in tumours while having lesser effects on the normal allele. These approaches will greatly improve our knowledge of the degree of selectivity of inhibitors that is needed, as well as the degree of knockdown of target activity required. However, siRNA approaches to targeting the PI3K pathway *in vivo* have not yet been reported. Further, it is important to emphasize that knockdown of a p110 or p85 isoform is likely to have very different consequences from chemical inhibition of that same isoform, because of the potential for compensation (knockdown) and dominant negative activity (drug).

AKT. As with inhibitors of PI3K that target the apex of the pathway, the theoretical advantage associated with AKT inhibition compared with downstream inhibition (for example, at mTOR) is that the PI3K/AKT pathway bifurcates and integrates with signals from other pathways as the signal is propagated (FIG. 1); the pathway can therefore be more globally and thereby effectively inhibited when targeting AKT (or indeed PI3K) directly. Theoretically, such inhibition might also be less susceptible to the complicating and still largely unknown effects of feedback loops than the inhibition of single branches further downstream. This efficacy is balanced by potential for toxicity and a narrow therapeutic index[128]. This might be particularly apparent with catalytic-domain inhibitors of AKT, which could demonstrate 'gain of function' activity. For this reason, a number of isoform-selective AKT catalytic-domain inhibitors, as well as inhibitors of the PH domain, are currently under development[129–131]. Whether these more selective inhibitors will demonstrate wider therapeutic indices is unknown. Recently, interest has focused on the alkylphosphocholines — miltefosine and perifosine — as AKT inhibitors that prevent membrane localization, possibly by interacting with the PH domain[132,133]. The specificity of these two drugs to the multiple PH domain-containing proteins is unknown at present but we have demonstrated selectivity to different PH domains. Whether this contributes to their apparently better therapeutic indices than other AKT inhibitors and relative lack of toxicity in ongoing clinical trials is under evaluation. Topical miltefosine has been approved for the treatment of cutaneous breast cancer metastases in Europe but cannot be formulated for parenteral use, and toxicity precludes oral use. In preclinical and early clinical trials, perifosine displayed antitumour activity[133]. Indeed, our preliminary results demonstrate a strong correlation between activity and target inhibition *in vivo* in tumours with aberrations in the PI3K pathway.

mTOR. The macrolide rapamycin, and its derivatives CCI-779 and RAD001, inhibit mTOR by binding to FK506-binding protein-12; however, they do not inhibit all functions of mTOR, nor do they inhibit the mTOR/RICTOR complex. These compounds have cytostatic activity as single agents *in vitro* and *in vivo* in animal models in haematological and solid tumours, and synergize with conventional chemotherapy and tamoxifen[134–137]. CCI-779 showed activity in Phase II clinical studies in patients with renal cell carcinoma and glioblastoma who have previously been treated with current standard therapies, and is being evaluated in combination with the aromatase inhibitor letrozole in metastatic breast cancer in a Phase III study[138–140]. The frequency of objective clinical and radiological tumour shrinkage is higher than expected from animal models but only occurs in a small portion of patients, emphasizing the need to develop approaches to identify patients likely to respond before the optimal utility of these drugs will be realized. In breast and

other cancer models *in vitro* and *in vivo*, it has been shown that reduced PTEN expression or expression of activated AKT confers susceptibility to inhibition of the PI3K/AKT pathway with mTOR inhibitors, which suggests that an optimal investigative strategy of the activity of these and other PI3K-pathway inhibitors in cancer might be to select patients whose tumours possess genomic anomalies resulting in PI3K-pathway

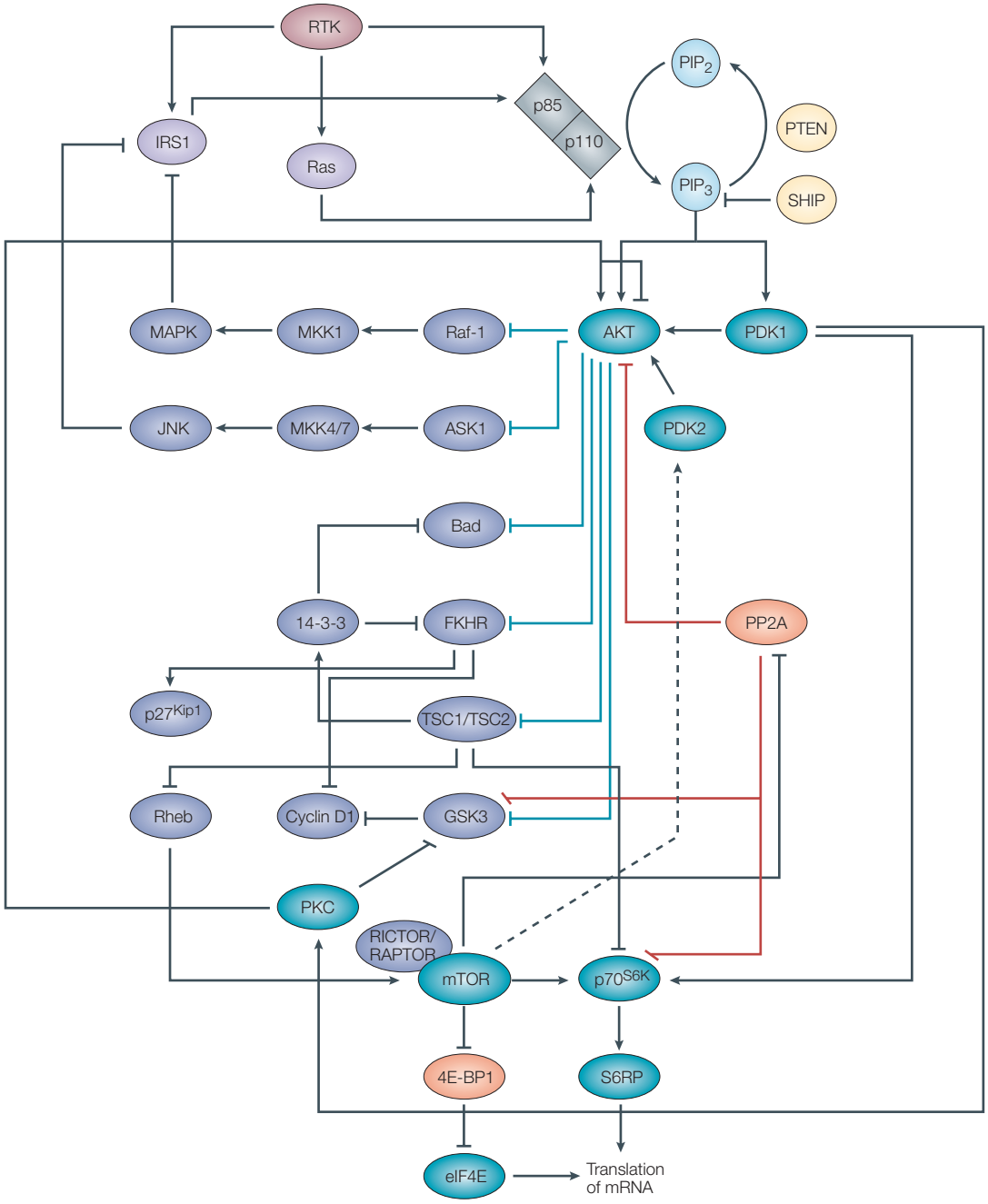


Figure 4 | **The application of mathematical modeling to the study of the phosphatidylinositol-3-kinase (PI3K)/AKT pathway.** Mathematical modeling allows detailed dissection of the interaction between individual pathway components and between these and components of other pathways such as the Ras/Raf/MAPK pathway. The dotted line indicates the recent demonstration that the mammalian target of rapamycin (mTOR)/RICTOR complex can phosphorylate AKT at serine 473 (that is, having PDK2 activity). mTOR, which can also complex with raptor, activates translation of messenger ribonucleic acid (mRNA) through a number of distinct mechanisms. 4E-BP1, 4E-binding protein 1; ASK1, apoptosis signal-regulating kinase 1; eIF4E, eukaryotic initiation factor 4E; FKHR, forkhead; GSK3, glycogen synthase kinase 3; IRS, insulin receptor substrate; PKC-protein kinase C; JNK-c, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKK, MAPK kinase; PDK, phosphoinositide dependent kinase; PIP_2, phosphatidylinositol-3,4-diphosphate; PIP_3, phosphatidylinositol-3,4,5-triphosphate; PP2A, protein phosphatase 2A; Rheb, atypical member of the Ras family with an unusually low intrinsic GTPase activity; RTK, receptor tyrosine kinase.

activation (such as *PIK3CA* mutation/amplification or *PTEN* loss)[141,142]. So far, however, the clinical development of mTOR inhibitors (and of perifosine) has lacked this specificity for clear and valid targets.

Rather than the PI3K/AKT pathway mediating single unidirectional regulation, the recent demonstration of the intricate role of mTOR in regulation of PI3K/AKT demonstrates that a detailed understanding of the PI3K pathway based on computational modelling will be necessary to determine the consequences of inhibition of particular nodes in the pathway (FIG. 4). We need to fully understand all activities of mTOR if we are to understand its validity as a target, the potential effects of its inhibition, and how best to target it. In addition, it is important to understand the specific functions of mTOR that are inhibited by rapalogs.

Other PI3K/AKT pathway components. A number of other targets in the PI3K pathway are under evaluation in preclinical models by both academia and drug companies (for example, ILK, PDK1, $p70^{S6K}$, in addition to Forkhead/FOXO1 activators)[143]. In addition, study of mechanisms of action of other drugs, which, like perifosine, were introduced without knowledge of their effects on the PI3K pathway, could uncover inhibitory activity; amiloride is a recent example[144]. Where these drugs and targets will fit into our armamentarium remains to be ascertained through preclinical studies as well as ongoing clinical trials.

Lessons learned from drugs targeting the PI3K pathway. Drugs targeted at molecules associated with PI3K signalling, such as EGFR, are in more advanced stages of development than direct inhibitors of the pathway. Importantly, it has been shown that PI3K/AKT pathway inhibition in response to targeting many of these molecules is necessary for therapeutic efficacy. This provides evidence of the central importance of the PI3K pathway to the function of these oncoproteins, and also that these validated targets signal through pathways utilized by the wild-type proteins in normal tissue. When developing these proteins as therapeutic targets, it is therefore an essential part of evaluation to monitor the activity of downstream PI3K/AKT-pathway effectors in response to the targeted therapy, both in terms of selecting patients likely to respond and also for the rapid identification of non-responders to allow triage to other therapeutic approaches. Trastuzumab, a monoclonal HER2 antibody, has dramatic efficacy in HER2-amplified breast cancer in the adjuvant and metastatic settings, particularly when combined with chemotherapy, and functional downregulation of PI3K/AKT is essential to its activity[145]. Coordinate development of trastuzumab with methods to identify patient tumours most likely to respond resulted in its efficient implementation into clinical practice. If the registration trials had not been restricted to patients with high-level expression of HER2, modelling approaches indicate that responses would have been sufficiently diluted to obscure trastuzumab activity, perhaps delaying or precluding its approval. This clarified an important paradigm for the development of targeted therapeutics: to coordinate validation of targets and drug development with implementation of molecular markers. This is now demonstrating importance in other diseases, with potential to triage CML and GIST patients to treatment with imatinib mesylate or related inhibitors based on the presence of a specific mutation, or the demonstration that the effects of erlotinib (Tarceva; Genentech) and gefitinib (Iressa; AstraZeneca) are most clearly manifest in lung cancer patients with mutations and/or amplification of *EGFR* resulting in downstream AKT phosphorylation (indicating functional activity of the genomic aberration). The role of assessment of PI3K-pathway activity might vary across tumour lineages, as it has been demonstrated that low baseline activity of AKT is associated with tumour responses to erlotinib in human glioblastoma[146–148]. In general, however, it seems that the identification of membrane receptor or other genomic anomalies of genes encoding tyrosine kinases associated with downstream AKT activation identifies tumours with a degree of 'oncogene addiction' that results in sensitivity to inhibition by kinase inhibitors or monoclonal antibodies; logically, then, downregulation of this AKT activation seems to parallel targeted therapy efficacy. AKT (re)activation in the face of a targeted therapy should therefore be evaluated to determine whether it signifies baseline or emergent resistance and should initiate the evaluation of combination therapy targeting the PI3K pathway as well as a search for mechanisms leading to target-independent activation of the PI3K pathway.

The failure of gefitinib, a small molecule with clear activity against a proportion of lung cancers with genomic anomalies of the *EGFR*, to demonstrate a survival advantage in unselected lung cancer patients stands in marked contrast to the novel paradigm of therapy development established by the introduction of trastuzumab, and reinforces the urgent need for target identification and development of molecular markers of drug efficacy[149–151]. Equally, although erlotinib has had somewhat more success recently in lung cancer, demonstration of the statistically significant though clinically modest benefit associated with its addition to gemcitabine (Gemzar; Lilly) for unselected patients with advanced pancreatic cancer has further underlined the need to identify accurate molecular markers to select patients likely to respond as well as indicators of efficacy of targeted therapies in specific cancer patients[152,153].

PI3K target identification and validation

PI3K/AKT anomalies in cancer constitute optimal targets for molecular therapeutics as they are common and dominantly dysregulate this oncogenic pathway[154–157]. However, improved understanding of this signalling network is necessary for efficient development and implementation of targeting strategies. Feedback loops must be comprehensively mapped to avoid unexpected effects of inhibition; secondary genomic changes (for example, with c-Kit in GIST tumours in response to imatinib) and 'nonspecific' stress-induced mechanisms

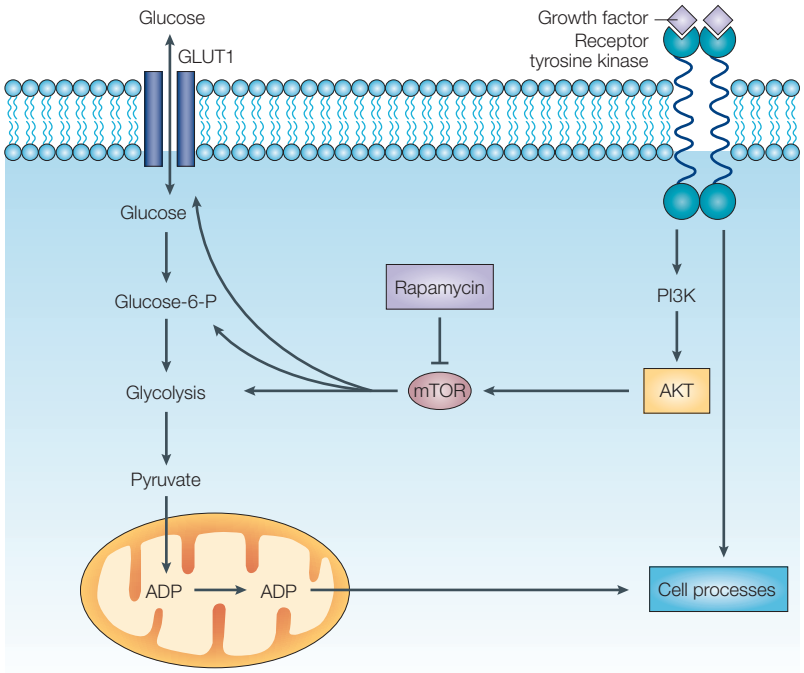


Figure 5 | **The role of AKT and mammalian target of rapamycin (mTOR) in glucose homeostasis.** AKT and mTOR are activated by membrane receptors and stimulate glycolysis in part by AKT-induced localization of the glucose transporters, including GLUT1 and GLUT3, to the cell surface, and maintenance of hexokinase function in the absence of extrinsic factors with resultant glucose production. PI3K, phosphatidylinositol-3-kinase.

triggered by pathway inhibitors must also be studied as potential mechanisms of resistance[158]. Targets must be identified and validated in clinical samples before investing time and money in developing a drug; *in vitro* and animal models provide useful information, but this information could prove misleading in terms of efficacy and toxicity, as well as the selection of important signalling nodes. Indeed, in the case of highly validated signalling nodes, human trials might be warranted even when modest or no activity is seen in animal models. For example, animal studies suggested that rapalogs would exhibit only cytostatic activity. Yet in a small subset of patients rapalogs demonstrate objective evidence of tumour shrinkage, suggesting that animal models do not necessarily reflect efficacy in selected human cancers.

In recent years, several groups have successfully integrated computational and mathematical modelling with experimental biology to identify systems properties of phosphoprotein signalling networks including the PI3K/AKT network (FIG. 4)[159–167]. Networks can be modelled at different levels, scales and detail using nonlinear coupled ordinary differential equation models, Bayesian networks, graph theory and re-write logic models. We are currently using this approach to allow the integration of knowledge of information flow through the PI3K pathway and other networks in normal and pathological states. Websites including the Signalling Gateway and the Nucleic Acids Research Database List (see Further information) use a similar approach. It is hoped that these maps or 'wiring diagrams' will lead to robust computational models (FIG. 4) of the pathway in normal and pathological states, and allow determination of key nodes in the network whose perturbation will have profound effects on signalling and tumorigenesis, yet with minimal toxicity.

The ideal target in a network is a key node essential to carcinogenesis ('oncogene addiction') but whose disruption will be tolerated, at least temporarily, by normal cells ('context-driven therapeutic index'). For example, significantly lower doses of imatinib mesylate are required for cytotoxicity in cells with the *BCR–ABL* translocation than in normal cells. However, key nodes in normal cells might be more difficult to exploit with an achievable therapeutic index (for example, AKT). As such, with an upregulated but structurally normal PI3K/AKT pathway in cancer cells, choosing to inhibit several peripheral targets might prove to be more effective and specific, while minimizing toxicity, than inhibiting a key central node. Nevertheless, in animal models, inhibition of the PI3K pathway at the peripheral site of mTOR with rapalogs has been found to block tumorigenesis induced via activation of multiple components of the pathway[168–170]. Each component of the pathway will therefore need to be assessed to evaluate its appropriateness as a target.

However, as evidenced by the efficacy of mTOR inhibitors in PTEN-deficient cells[171], of imatinib in CML and GIST tumours[172,173], and of trastuzumab in HER2-amplified breast cancer, the presence of a genomically anomalous target resulting in activation of the PI3K/AKT pathway at the proteomic level in cancer cells indicates a likelihood that the tumour has developed a degree of 'oncogene addiction' and is in part dependent on the pathway, which provides a good starting point for drug development and PI3K/AKT target exploitation.

We will also need to combine assessment of clinical outcomes with assessment of molecular markers as a means of determining the degree and duration of target 'knockdown' that results in optimal clinical efficacy — that is, the biologically relevant dose. The current method of driving Phase I dose-finding trials to toxicity — that is, maximum tolerated dose (MTD) — is likely to be inappropriate with novel targeted therapies and must be changed if we are to realize the full potential of this approach. We feel that the 'biologically relevant dose' should become the major driver of dose level and schedule determination, and, indeed, of Phase I trials. The currently used concept of 'therapeutic index' in the clinical development of drugs at the MTD is more suited to nonspecific cytotoxics than to 'targeted therapeutics'. As we develop molecular diagnostic technologies to find early biological correlates of the clinical efficacy of a novel drug by studying the target, its pathway and other related molecules, it might become possible to use these correlates as an early surrogate for clinical benefit and also to triage non-responders to appropriate therapy earlier. With mTOR inhibitors, we could assess activity in terms of downregulation of phosphorylation of proteins, including $p70^{S6K}$, and

functionally in terms of cell proliferation. Indeed, several studies suggest that this can be extended to surrogate tissues, with knockdown of mTOR signalling by rapalogs in peripheral blood lymphocytes or inhibition of EGFR and downstream target phosphorylation in skin by EGFR inhibitors accurately reflecting target knockdown in tumour. It is important to emphasize that target knockdown, while required for efficacy, is not sufficient for efficacy if a tumour is not 'addicted' to the targeted pathway or if a downstream activation event bypasses the targeted therapeutic. Initially, it will be necessary to correlate biological readouts with antitumour activity to determine the appropriate magnitude and duration of target inhibition and to validate surrogate markers of efficacy.

The clinical development of inhibitors of the PI3K/AKT pathway with rational targets in mind (for example, activating genomic anomalies including *PTEN* loss[171] and *PIK3CA* amplification/mutation[174]) in combination with downstream molecular marker evaluation is much more likely to yield success than current approaches. There are, however, two other possible approaches to the introduction of inhibitors of the PI3K pathway into clinical trials. One is the detection of activation of the PI3K pathway using proteomic technologies such as western blotting or REVERSE PHASE PROTEIN ARRAYS as a surrogate marker for probable activating genomic anomalies, although this is likely to be somewhat less specific to potential drug efficacy than the identification of activating genomic anomalies themselves. The other approach would be inclusion of all patients in molecular marker-driven trials with mandatory tissue biopsies and retrospective determination of markers of early response by molecular study of responding tumours. In this case, careful patient sample collection and analysis is essential, particularly in responders. This approach is probably most appropriate with drugs when understanding of likely markers of efficacy from preclinical studies is limited.

Even when a valid target is present, the tumour might be resistant to therapy. If the targeted anomaly (for example, activating mutation) is an early event, other genetic changes can subsequently occur that lessen or modulate a tumours reliance on the original anomaly. Trastuzumab responses in HER2-amplified breast cancers are generally under 50%, as the activity of trastuzumab is modulated by PTEN, in turn regulated by mediators such as SRC, insulin-like growth factor (IGF)-I and potentially other molecules[175,176]. mTOR inhibition is now being explored in an attempt to overcome trastuzumab resistance caused by downregulated PTEN. However, this could, paradoxically, activate PI3K and AKT. This emphasizes the importance of fully understanding the 'wiring' of the signalling pathway downstream from a target. High-throughput proteomic technologies will help us validate targets in a way not possible by looking only at the genome. Further, as the study of secondary mutations resulting in imatinib resistance has shown us, when rational 'driving' oncogenic targets are exploited with novel therapies, the emergence of resistance is likely in many cases to be a relatively predictable event that results from reactivating the same oncogenic pathway that mediated the effects of the primary oncogenic anomaly. The development or selection of these mutations clearly validates the target and emphasizes the need for the development of 'follow on' drugs able to target these selected or induced mutations.

REVERSE PHASE PROTEIN ARRAY
An array that immobilizes the whole repertoire of patient proteins that represent the state of individual tissue cell populations undergoing disease transitions.

Imaging the PI3K pathway

Tumour biopsies from patients on therapy can be difficult to obtain technically and ethically. Therefore non- or minimally invasive approaches to determine the effects of targeted therapeutics could have broad utility. AKT is stimulated by the insulin receptor, which is involved in regulation of glucose metabolism, and might in part explain the Warburg effect (the metabolic shift from oxidative to elevated anaerobic glycolysis in cancer cells) (FIG. 5)[177,178]. AKT-induced localization of glucose transporters, including GLUT1 and GLUT3, to the cell surface and maintenance of hexokinase function in the absence of extrinsic factors might contribute to the increased ability of tumour cells to utilize external nutrients[179]. The shift to glycolytic metabolism after neoplastic transformation is now exploited for fluorodeoxyglucose positron emission tomography (FDG–PET) imaging[180]. Indeed, changes in FDG–PET are predictive of response to imatinib mesylate in GIST as early as 72 hours after its initiation, long before other technologies such as computed tomography can detect response. Whether this reflects imatinib effects on tumour metabolism in general or a more specific effect due to interactions between *BCR–ABL* and the PI3K pathway remains to be determined. As the PI3K pathway directly targets glucose uptake and metabolism, FDG–PET is likely to prove particularly useful in functional imaging of the efficacy of inhibitors of the PI3K pathway. Based on the importance of this pathway, a number of groups are developing imaging agents designed to detect the activation status of components of the PI3K pathway such as p110 and AKT directly. Molecular imaging therefore has the potential to demonstrate early target inhibition. This approach could achieve optimal efficacy if combined with imaging of functional outcomes, such as changes in cell proliferation, vascularity or apoptosis, all of which could be useful as surrogates of tumour response.

Conclusions

One major foreseen difficulty with the development and implementation of targeted therapies is the prevailing belief that most solid tumours do not have single, dominant oncogenic abnormalities that can be targeted with high efficiency. It has therefore been theorized that the best we can achieve with most targeted therapies alone in solid tumours are partial responses or potentially stable disease. Opponents of this theory point to GIST tumours, which acquire a number of genetic abnormalities as they progress but still retain sensitivity to imatinib mesylate, or to lung cancer with multiple genetic aberrations that respond

Box 1 | **Considerations for development of PI3K inhibitors**

New strategies need to be implemented to coordinate target validation, preclinical and early clinical evaluation for the successful and timely development of phosphatidylinositol-3-kinase (PI3K) inhibitors (see figure).

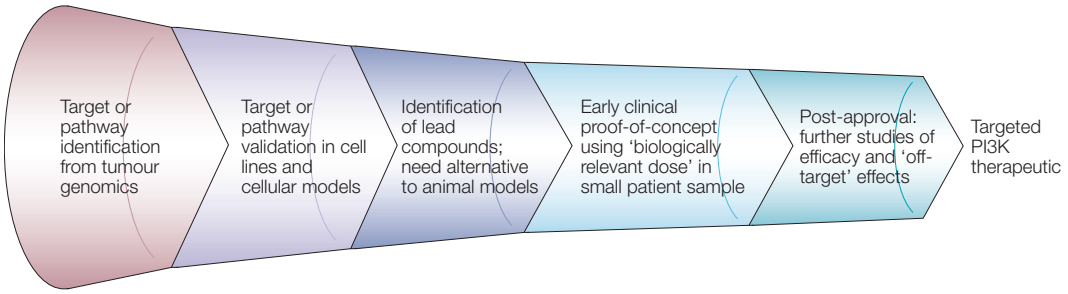
First, following the identification of a valid high-quality target in cancer cells, tools such as chemical genomics or small interfering RNA (siRNA) should be used concomitantly with high-throughput genomic and proteomic studies to determine on- and off-target effects of inhibition and pathway crosstalk. Although siRNA target validation offers specific and effective knockdown, this might not mimic the effects of drugs where the outcome is an inhibited molecule with potential novel or other functions.

Second, methods to combine therapeutic approaches, including signal transduction inhibitors, radiation or chemotherapy, should be sought earlier in development. As the PI3K pathway can mediate resistance to various therapeutic agents and protects cells from the consequences of stress, combinations of PI3K inhibitors with other therapies need exploration, particularly in the presence of activating PI3KCA or deleterious PTEN mutations.

Third, modulating tumour-specific targets or anomalies such as PI3KCA or PTEN mutations will probably minimize the dose of drug required for molecular and antitumour efficacy with resultant minimal toxicity — a concept that can be termed context-driven therapeutic index. However, current animal models might prove non-predictive of efficacy or toxicity and the development of more appropriate tumour models driven by specific genetic aberrations will be necessary. For very high-quality targets, evaluation in human patients without animal data might also be an option.

Fourth, mandated tumour biopsies in molecular-marker-driven trials will be necessary for the efficient evaluation of novel targeted therapeutics. It will be crucial to distinguish on-target from off-target activity to prevent a good target being discarded due to a bad drug. Moreover, biological effects must always be correlated with clinical efficacy. To address this, we must begin to employ the concept of 'biologically relevant dose' rather than maximum tolerated dose (MTD) to guide drug schedules; this might make it feasible to merge the different trial phases (I, II and III) into one continuous clinical–biological assessment of drug efficacy in patients. In particular, the goals of Phase I trials will need to be revised if we are no longer conducting drug testing driven by MTD. Further, large, randomized Phase III trials are probably only necessary when assessing drugs that benefit a small proportion of patients with a particular cancer where no validated mechanism exists to separate potential responders from non-responders.

Finally, after approval, studies must continue to improve the identification of likely responders, to facilitate early identification of patients who are not responding, to identify the spectrum of tumours and molecular aberrations likely to benefit, and to monitor off-target effects of the drug. Emergent resistance in tumours possessing the validated target(s) should initiate biological studies to determine molecular mechanism(s) likely to be predictable secondary events in many cases.



to erlotinib and gefitinib. So despite the occurrence of subsequent genetic events, solid tumours can retain sensitivity ('addiction') to early abnormalities. In some cases, later events can promote a growth advantage only in the presence of initial changes and correction of the latter might remove this growth advantage. Tumours with p53 mutations tolerate retinoblastoma mutations, but restoration of wild-type p53 makes retinoblastoma mutations pro-apoptotic[181]. It is therefore important to understand the mechanisms of carcinogenesis in specific tumours to identify potentially important early changes. Hereditary cancers could also provide clues. Such knowledge is now being exploited in renal cell carcinoma with early somatic *vHL* gene changes[182].

Activating PI3K/AKT pathway anomalies, such as PIK3CA mutations or PTEN loss/downregulation, are likely to constitute an excellent mechanism to identify patients likely to respond to PI3K pathway inhibitors. The clinical development of PI3K/AKT pathway inhibitors using such molecular markers is therefore much more likely to yield success than current approaches. There is much evidence that PI3K/AKT signalling mediates resistance to both nonspecific and targeted cancer therapies. There might therefore be benefits to combining these therapies with PI3K inhibitors, particularly in the presence of genomic anomalies that activate the PI3K pathway. When incorporating inhibitors of the PI3K/AKT pathway into studies to investigate their potential clinical utility, the MTD-based approach to drug development and the traditional model of sequential Phase I, II and III trials will probably not be appropriate and will delay the opportunity

Table 4 | **Abnormalities in pathways related to the PI3K/AKT signalling pathway in cancer**

Molecule	Alteration in tumours	Frequency	Tumour lineage
EGFRvIII	Alternate splicing Deletion AA 6-273	>80% >50% 40%	Glioma Breast Non-small-cell lung cancer
EGFR	Activating mutations	10%	Lung cancer (Asian, women, non- smokers), colorectal (rare)
EGFR	Amplification	Variable	Breast, lung, colorectal, glioblastoma
HER2/*neu*	Amplification	30% 8%	Breast cancer Ovarian cancer
HER2/*neu*	Mutation	10%	Lung adenocarcinoma
BCR–ABL	Translocation	>90%	CML
Kit	Mutation	>80%	GIST
Ras	Mutation	>90% ~30% 5%	Pancreatic cancer Lung cancer and in surrounding 'normal' lung epithelium in up to 30% of lung cancer patients Breast
bRaf	Mutation	Variable	Up to 66% of malignant melanomas Less common in other tumours, for example, lung
PDGFR	Mutation	Rare >80%	GIST Dermatofibrosarcoma protuberans Hypereosinophilic syndrome
ER	Expression	~70%	Breast
Src	Mutation Activation	Unclear >50% 50%	Bowel Bowel Breast
Integrins	Increased expression	Variable	Multiple

CML, chronic myeloid leukaemia; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; GIST, gastrointestinal stromal tumour; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol-3-kinase.

to identify effective therapeutic approaches. BOX 1 details an emerging approach with strong support from recent clinical trials, which could allow us to better and more efficiently exploit the potential benefits of targeted therapies. Finally, there is concern regarding the lack of truly relevant and representative *in vitro* and animal models for predicting *in vivo* toxicity and efficacy, and for validation of imaging modalities. Cautious and intelligent selection of animal models and interpretation of the data they generate will be required. In an era of rapidly emerging technologies with the capability of defining tumours at a molecular level, perhaps it soon will be the time, using available small molecules, antibodies and RNA interference technology, to drive translation of approaches to human trials of biologically relevant dose rather than MTD, therefore fulfilling the promise of personalized molecular medicine.

1. Fruman, D. A., Meyers, R. E. & Cantley, L. C. Phosphoinositide kinases. *Annu. Rev. Biochem*. **67**, 481–507 (1998). **Excellent review of the structure and function of PI3Ks.**
2. Hiles, I. D. *et al*. Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. *Cell* **70**, 419–429 (1992).
3. Jimenez, C. *et al*. Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. *EMBO J.* **17**, 743–753 (1998).
4. Pawson, T. & Nash, P. Protein–protein interactions define specificity in signal transduction. *Genes. Dev.* **14**, 1027–1047 (2000).
5. Davies, S. P., Reddy, H., Caivano, M. & Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **351**, 95–105 (2000).
6. Oudit, G. Y. *et al*. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. *J. Mol. Cell Cardiol*. **37**, 449–471 (2004).
7. Bi, L., Okabe, I., Bernard, D. J., Wynshaw-Boris, A. & Nussbaum, R. L. Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110α subunit of phosphoinositide 3-kinase. *J. Biol. Chem*. **274**, 10963–10968 (1999).
8. Brachmann, S. M., Ueki, K., Engelman, J. A., Kahn, R. C. & Cantley, L. C. Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. *Mol. Cell. Biol.* **25**, 1596–1607 (2005).
9. Okkenhaug, K. *et al*. Impaired B and T cell antigen receptor signaling in p110δ PI 3-kinase mutant mice. *Science* **297**, 1031–1034 (2002).
10. Bilancio, A. *et al*. Key role of the p110δ isoform of PI3K in B cell antigen and IL4 receptor signalling- comparative analysis of genetic and pharmacological interference with p110δ function in B cells. *Blood.* 2005 Sep 22; [Epub ahead of print].
11. Northcott, C. A. *et al*. Upregulated function of phosphatidylinositol-3-kinase in genetically hypertensive rats: a moderator of arterial hypercontractility. *Clin. Exp. Pharmacol. Physiol.* **32**, 851–858 (2005).
12. Condliffe, A. M. *et al*. Sequential activation of class IB and class IA PI3K is important for the primed respiratory burst of human but not murine neutrophils. *Blood* **106**, 1432–1440 (2005).
13. Sujobert, P. *et al*. Essential role for the p110δ isoform in phosphoinositide 3-kinase activation and cell proliferation in acute myeloid leukemia. *Blood* **106**, 1063–1066 (2005).
14. Yip, S. C. *et al*. Over-expression of the p110β but not p110α isoform of PI 3-kinase inhibits motility in breast cancer cells. *Cell Motil. Cytoskeleton* **59**, 180–188 (2004).
15. Geng, L. *et al*. A specific antagonist of the p110δ catalytic component of phosphatidylinositol 3'-kinase, IC486068, enhances radiation-induced tumor vascular destruction. *Cancer Res*. **64**, 4893–4899 (2004)
16. Campbell, M. *et al*. Glucose-potentiated chemotaxis in human vascular smooth muscle is dependent on cross-talk between the PI3K and MAPK signaling pathways. *Circ. Res*. **95**, 380–388 (2004).
17. Gukovsky, I. *et al*. Phosphatidylinositide 3-kinase γ regulates key pathologic responses to cholecystokinin in pancreatic acinar cells. *Gastroenterology* **126**, 554–566 (2004).
18. Leverrier, Y. *et al*. Class I phosphoinositide 3-kinase p110β is required for apoptotic cell and Fcγ receptor-mediated phagocytosis by macrophages. *J. Biol. Chem*. **278**, 38437–38442 (2003).
19. Chantry, D. *et al*. p110δ, a novel phosphatidylinositol 3-kinase catalytic subunit that associates with p85 and is expressed predominantly in leukocytes. *J. Biol. Chem*. **272**, 19236–19241 (1997).

20. Perrino, C. *et al*. Targeted inhibition of β-adrenergic receptor kinase-1-associated phosphoinositide-3 kinase activity preserves β-adrenergic receptor signaling and prolongs survival in heart failure induced by calsequestrin overexpression. *J. Am. Coll. Cardiol*. **45**, 1862–1870 (2005).
21. Jackson, S. P. *et al*. PI3-kinase p110β: a new target for antithrombotic therapy. *Nature Med.* **11**, 507–514 (2005).
22. Coffer, P. J. & Woodgett, J. R. Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. *Eur. J. Biochem.* **201**, 475–81 (1991).
23. 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**, 274–77 (1991).
24. Murthy, S. S., Tosolini, A., Taguchi, T. & Testa, J. R. Mapping of AKT3, encoding a member of the Akt/protein kinase B family, to human and rodent chromosomes by fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.* **88**, 39–40 (2000).
25. Alessi, D. R. *et al*. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J.* **15**, 6541–551 (1996).
26. Blume-Jensen, P. & Hunter, T. Oncogenic kinase signalling. *Nature* **411**, 355–65 (2001).
27. Lynch, D. K., Ellis, C. A., Edwards, P. A. & Hiles, I. D. Integrin-linked kinase regulates phosphorylation of serine 473 of protein kinase B by an indirect mechanism. *Oncogene* **18**, 8024–32 (1999).
28. Kawakami, Y. *et al*. Protein kinase C βII regulates Akt phosphorylation on Ser-473 in a cell type- and stimulus-specific fashion. *J. Biol. Chem.* **279**, 47720–5 (2004).
29. Sarbassov dos, D., Guertin, D. A., Ali, S. M. & Sabatini, D. M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**, 1098–101 (2005).
30. Arboleda, M. J. *et al*. Overexpression of AKT2/protein kinase Bβ leads to up-regulation of β_1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells. *Cancer Res*. **63**, 196–206 (2003).
31. Yang, Z. Z. *et al*. Protein kinase B α/Akt1 regulates placental development and fetal growth. *J. Biol. Chem*. **278**, 32124–31 (2003).
32. Peng, X. D. *et al*. Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. *Genes Dev.* **17**, 1352–65 (2003).
33. Cho, H., Thorvaldsen, J. L., Chu, Q., Feng, F. & Birnbaum, M. J. Akt1/PKBα is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J. Biol. Chem.* **276**, 38349–52 (2001).
34. Testa, J. R. & Bellacosa, A. AKT plays a central role in tumorigenesis. *Proc. Natl Acad. Sci. USA* **98**, 10983–985 (2001).
35. Vivanco, I. & Sawyers, C. L. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nature Rev. Cancer* **2**, 489–501 (2002).
36. Luo, J., Manning, B. D. & Cantley, L. C. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. *Cancer Cell* **4**, 257–62 (2003).
37. Chen, X. *et al*. Constitutively active Akt is an important regulator of TRAIL sensitivity in prostate cancer. *Oncogene* **20**, 6073–83 (2001).
38. Wang, Q. *et al*. Regulation of TRAIL expression by the phosphatidylinositol 3-kinase/Akt/GSK-3 pathway in human colon cancer cells. *J. Biol. Chem.* **277**, 36602–610 (2002).
39. Carroll, P. E. *et al*. Centrosome hyperamplification in human cancer: chromosome instability induced by p53 mutation and/or Mdm2 overexpression. *Oncogene* **18**, 1935–44 (1999).
40. Toi, M., Saji, S., Suzuki, A., Yamamoto, Y., Tominaga, T. MDM2 in Breast Cancer. *Breast Cancer* **25**, 264–68 (1997).
41. Sherr, C. J. & Weber, J. D. The ARF/p53 pathway. *Curr. Opin. Genet. Dev.* **10**, 94–99 (2000).
42. Brunet, A. *et al*. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96**, 857–68 (1999).
Excellent review of some of the downstream mechanisms of AKT action.
43. Cahill, C. M. *et al*. Phosphatidylinositol 3-kinase signaling inhibits DAF-16 DNA binding and function via 14–3-3-dependent and 14–3-3-independent pathways. *J. Biol. Chem.* **276**, 13402–10 (2001).
44. Wendel, H. G. *et al*. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* **428**, 332–337 (2004).
45. Schmelzle, T. & Hall, M. N. TOR, a central controller of cell growth. *Cell* **103**, 253–262 (2000).
Excellent review of mTOR and its activity.
46. Lazaris-Karatzas, A., Montine, K. S. & Sonenberg, N. Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5' cap. *Nature* **345**, 544–547 (1990).
47. Polunovsky, V. A. *et al*. Translational control of the antiapoptotic function of Ras. *J. Biol. Chem.* **275**, 24776–24780 (2000).
48. Potter, C. J., Pedraza, L. G. & Xu, T. Akt regulates growth by directly phosphorylating Tsc2. *Nature Cell Biol.* **4**, 658–665 (2002).
49. Tee, A. R. *et al*. Tuberous sclerosis complex-1 and-2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *Proc. Natl Acad. Sci. USA* **99**, 13571–576 (2002).
50. Dan, H. C. *et al*. Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. *J. Biol. Chem.* **277**, 35364–35370 (2002).
51. Hengstschlager, M., Rosner, M., Fountoulakis, M. & Lubec, G. Tuberous sclerosis genes regulate cellular 14-3-3 protein levels. *Biochem. Biophys. Res. Commun.* **312**, 676–83 (2003).
52. Harrington, L. S. *et al*. The TSC1–2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J. Cell Biol.* **166**, 213–23 (2004).
53. Zhang, H. *et al*. Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K-Akt signaling through downregulation of PDGFR. *J. Clin. Invest*. **112**, 1223–1233 (2003).
54. Sun, S. Y. *et al*. Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Res.* **65**, 7052–7058 (2005).
55. Hay, N. The Akt-mTOR tango and its relevance to cancer. *Cancer Cell*. **8**, 179–183 (2005).
56. Hardie, D. G., Scott, J. W., Pan, D. A. & Hudson, E. R. Management of cellular energy by the AMP-activated protein kinase system. *FEBS Lett*. **546**, 113–120 (2003).
57. Shaw, R. J. *et al*. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc. Natl Acad. Sci. USA* **101**, 3329–3335 (2004).
58. Shaw, R. J. *et al*. The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* **6**, 91–99 (2004).
59. Fujita, N., Sato, S. & Tsuruo, T. Phosphorylation of p27Kip1 at threonine 198 by p90 ribosomal protein S6 kinases promotes its binding to 14-3-3 and cytoplasmic localization. *J. Biol. Chem.* **278**, 49254–49260 (2003).
60. Shin, I. *et al*. PKB/Akt mediates cell-cycle progression by phosphorylation of $p27^{Kip1}$ at threonine 157 and modulation of its cellular localization. *Nature Med.* **8**, 1145–1152 (2002).
61. Zhou, B. P. *et al*. Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. *Nature Cell Biol.* **3**, 245–252 (2001).
62. Diehl, J. A., Cheng, M., Roussel, M. F. & Sherr, C. J. Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev.* **12**, 3499–3511 (1998).
63. Kim, W. Y. & Kaelin, W. G. Role of VHL gene mutation in human cancer. *J. Clin. Oncol.* **22**, 4991–5004 (2004).
64. Skinner, H. D., Zheng, J. Z., Fang, J., Agani, F. & Jiang, B. H. Vascular endothelial growth factor transcriptional activation is mediated by hypoxia-inducible factor 1α, HDM2, and p70S6K1 in response to phosphatidylinositol 3-kinase/AKT signaling. *J. Biol. Chem.* **279**, 45643–51 (2004).
65. Zhang, D. & Brodt, P. Type 1 insulin-like growth factor regulates MT1-MMP synthesis and tumor invasion via PI 3-kinase/Akt signaling. *Oncogene* **22**, 974–82 (2003).
66. Kimura, A. *et al*. Induction of hTERT expression and phosphorylation by estrogen via Akt cascade in human ovarian cancer cell lines. *Oncogene* **23**, 4505–15 (2004).
67. Remy, I., Montmarquette, A. & Michnick, S. W. PKB/Akt modulates TGF-β signalling through a direct interaction with Smad3. *Nature Cell Biol.* **6**, 358–65 (2004).
68. Shou, J. *et al*. Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J. Natl Cancer Inst.* **96**, 926–35 (2004).
69. Mondesire, W. H. *et al*. Targeting mammalian target of rapamycin synergistically enhances chemotherapy-induced cytotoxicity in breast cancer cells. *Clin. Cancer Res.* **10**, 7031–42 (2004).
70. Knuefermann, C. *et al*. HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. *Oncogene* **22**, 3205–12 (2003).
71. Gupta, A. K. *et al*. Radiation sensitization of human cancer cells *in vivo* by inhibiting the activity of PI3K using LY294002. *Int. J. Radiat. Oncol. Biol. Phys.* **56**, 846–53 (2003).
72. Brognard, J., Clark, A. S., Ni, Y. & Dennis, P. A. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. *Cancer Res.* **61**, 3986–97 (2001).
73. Kim, D. *et al*. AKT/PKB signaling mechanisms in cancer and chemoresistance. *Front Biosci.* **10**, 975–87 (2005).
74. Stoica, G. E. *et al*. Effect of estradiol on estrogen receptor-α gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway. *Oncogene* **22**, 7998–8011 (2003).
75. Mendez, P., Azcoitia, I. & Garcia-Segura, L. M. Estrogen receptor α forms estrogen-dependent multimolecular complexes with insulin-like growth factor receptor and phosphatidylinositol 3-kinase in the adult rat brain. *Brain Res. Mol. Brain Res.* **112**, 170–6 (2003).
76. Nagata, Y. *et al*. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* **6**, 117–127 (2004).
Excellent review of the role of PTEN in trastuzumab resistance in breast cancer.
77. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
Excellent review of neoplastic progression due to the collective role of multiple genetic and molecular abnormalities.
78. Bachman, K. E. *et al*. The PIK3CA Gene is Mutated with High Frequency in Human Breast Cancers. *Cancer Biol. Ther.* **3**, 772–775 (2004).
79. Kang, S., Bader, A. G. & Vogt, P. K. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc. Natl Acad. Sci. USA* **102**, 802–807 (2005).
Excellent paper which demonstrates that PI3K mutations are oncogenic in human cancers.
80. Samuels, Y. *et al*. High frequency of mutations of the PIK3CA gene in human cancers. *Science* **304**, 554 (2004).
81. Campbell, I. G. *et al*. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res.* **64**, 7678–7681 (2004).
82. Shayesteh, L. *et al*. PIK3CA is implicated as an oncogene in ovarian cancer. *Nature Genet.* **21**, 99–102 (1999).
83. Woenckhaus, J. *et al*. Genomic gain of PIK3CA and increased expression of p110α are associated with progression of dysplasia into invasive squamous cell carcinoma. *J. Pathol.* **198**, 335–342 (2002).
84. Ma, Y. Y. *et al*. PIK3CA as an oncogene in cervical cancer. *Oncogene* **19**, 2739–2744 (2000).
85. Mizoguchi, M., Nutt, C. L., Mohapatra, G. & Louis, D. N. Genetic alterations of phosphoinositide 3-kinase subunit genes in human glioblastomas. *Brain Pathol.* **14**, 372–377 (2004).
86. Broderick, D. K. *et al*. Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. *Cancer Res.* **64**, 5048–5050 (2004).
87. Jucker, M. *et al*. Expression of a mutated form of the p85α regulatory subunit of phosphatidylinositol 3-kinase in a Hodgkin's lymphoma-derived cell line (CO). *Leukemia* **16**, 894–901 (2002).
88. Cheng, J. Q. *et al*. Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc. Natl Acad. Sci. USA* **93**, 3636–3341 (1996).
89. Ruggeri, B. A., Huang, L., Wood, M., Cheng, J. Q. & Testa, J. R. Amplification and overexpression of the AKT2 oncogene in a subset of human pancreatic ductal adenocarcinomas. *Mol. Carcinog.* **21**, 81–86 (1998).
90. Bellacosa, D. *et al*. Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. *Int. J. Cancer* **64**, 280–285 (1995).
91. Staal, S. P. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc. Natl Acad. Sci. USA* **84**, 5034–5037 (1987).
92. Eng, C. PTEN: one gene, many syndromes. *Hum. Mutat.* **22**, 183–198 (2003).
93. Li, Y. L., Tian, Z., Wu, D. Y., Fu, B. Y. & Xin, Y. Loss of heterozygosity on 10q23. 3 and mutation of tumor suppressor gene PTEN in gastric cancer and precancerous lesions. *World J. Gastroenterol.* **11**, 285–288 (2005).
94. Li, J. *et al*. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275**, 1943–1947 (1997).

95. Cairns, P. *et al*. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res*. **57**, 4997–5000 (1997).
96. Garcia, J. M. *et al*. Allelic loss of the PTEN region (10q23) in breast carcinomas of poor pathophenotype. *Breast Cancer Res. Treat.* **57**, 237–243 (1999).
97. Khan, S. *et al*. PTEN promoter is methylated in a proportion of invasive breast cancers. *Int. J. Cancer* **112**, 407–410 (2004).
98. Goel, A. *et al*. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. *Cancer Res.* **64**, 3014–3021 (2004).
99. Stephens, P. *et al*. A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. *Nature Genet.* **37**, 590–592 (2005).
100. Davies, H. *et al*. Somatic mutations of the protein kinase gene family in human lung cancer. *Cancer Res.* **65**, 7591–7595 (2005).
101. Parsons, D. W. *et al*. Colorectal cancer: mutations in a signalling pathway. *Nature* **436**, 792 (2005).
102. Wang, Z. *et al*. Mutational analysis of the tyrosine phosphatome in colorectal cancers. *Science.* **304**, 1164–1166 (2004).
103. Rodriguez-Viciana, P. *et al*. Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. *Cell* **89**, 457–467 (1997).
104. Almoguera, C. *et al*. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell* **53**, 549–554 (1988).
105. Yanez, L., Groffen, J. & Valenzuela, D. M. c-K-ras mutations in human carcinomas occur preferentially in codon 12. *Oncogene* **1**, 315–318 (1987).
106. Nelson, M. A., Wymer, J. & Clements, N. Jr. Detection of K-ras gene mutations in non-neoplastic lung tissue and lung cancers. *Cancer Lett* **103**, 115–121 (1996).
107. Davies, H. A. *et al*. Mutations of the BRAF gene in human cancer. *Nature.* **417**, 949–954 (2002).
108. Rolitsky, C. D., Theil, K. S., McGaughy, V. R., Copeland, L. J. & Niemann, T. H. HER-2/neu amplification and overexpression in endometrial carcinoma. *Int. J. Gynecol. Pathol.* **18**, 138–143 (1999).
109. Slamon, D. J. *et al*. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244**, 707–712 (1989).
110. Stephens, P. *et al*. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature* **431**, 525–526 (2004).
Excellent paper which demonstrates that HER2 mutations occur in 10% human lung cancers
111. Klos, K. S. *et al*. Combined trastuzumab and paclitaxel treatment better inhibits ErbB-2-mediated angiogenesis in breast carcinoma through a more effective inhibition of Akt than either treatment alone. *Cancer* **98**, 1377–1385 (2003).
112. Nicholson, K. M., Streuli, C. H. & Anderson, N. G. Autocrine signalling through erbB receptors promotes constitutive activation of protein kinase B/Akt in breast cancer cell lines. *Breast Cancer Res. Treat.* **81**, 117–128 (2003).
113. Ekstrand, A. J., Sugawa, N., James, C. D. & Collins, V. P. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. *Proc. Natl Acad. Sci. USA* **89**, 4309–4313 (1992).
114. Sordella, R., Bell, D. W., Haber, D. A. & Settleman, J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* **305**, 1163–1167 (2004).
115. Tang, C. K., Gong, X. Q., Moscatello, D. K., Wong, A. J. & Lippman, M. E. Epidermal growth factor receptor vIII enhances tumorigenicity in human breast cancer. *Cancer Res.* **60**, 3081–3087 (2000).
116. Barber, T. D., Vogelstein, B., Kinzler, K. W. & Velculescu, V. E. Somatic mutations of EGFR in colorectal cancers and glioblastomas. *N. Engl. J. Med*. **351**, 2883 (2004).
117. Okamoto, I. *et al*. Expression of constitutively activated EGFRvIII in non-small cell lung cancer. *Cancer Sci*. **94**, 50–56 (2003).
118. Rosenzweig, K. E., Youmell, M. B., Palayoor, S. T. &Price, B. D. Radiosensitization of human tumor cells by the phosphatidylinositol-3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G2-M delay. *Clin. Cancer Res.* **3**, 1149–1156 (1997).
119. Ng, S. S., Tsao, M. S., Nicklee, T. & Hedley, D. W. Wortmannin inhibits pkb/akt phosphorylation and promotes gemcitabine antitumor activity in orthotopic human pancreatic cancer xenografts in immunodeficient mice. *Clin. Cancer Res.* **7**, 3269–3275 (2001).
120. Kim, S. H. *et al*. Potentiation of chemosensitivity in multidrug-resistant human leukemia CEM cells by inhibition of DNA-dependent protein kinase using wortmannin. *Leuk. Res.* **24**, 917–925 (2000).
121. Schultz, R. M. *et al*. *In vitro* and *in vivo* antitumor activity of the phosphatidylinositol-3-kinase inhibitor, wortmannin. *Anticancer Res.* **15**, 1135–1139 (1995).
122. Hu, L., Zaloudek, C., Mills, G. B., Gray, J. & Jaffe, R. B. *In vivo* and *in vitro* ovarian carcinoma growth inhibition by a phosphatidylinositol 3-kinase inhibitor (LY294002). *Clin. Cancer Res.* **6**, 880–886 (2000).
123. Hu, L., Hofmann, J., Lu, Y., Mills, G. B. & Jaffe, R. B. Inhibition of phosphatidylinositol 3 -kinase increases efficacy of paclitaxel in *in vitro* and *in vivo* ovarian cancer models. *Cancer Res.* **62**, 1087–1092 (2002).
124. Shokat, K. *et al*. Isoform-specific phosphoinositide 3-kinase inhibitors from an arylmorpholine scaffold. *Bioorg. Med. Chem*. **12**, 4749–4759 (2004).
125. Ward, S. G. & Finan, P. Isoform-specific phosphoinositide 3-kinase inhibitors as therapeutic agents. *Curr. Opin. Pharmacol.* **3**, 426–434 (2003).
126. Wymann, M. P., Zvelebil, M. & Laffargue, M. Phosphoinositide 3-kinase signalling — which way to target?, *Trends. Pharmacol. Sci.* **24**, 366–376 (2003).
127. Roberson, A., Jackson, S., Kenche, V., Yaip, C., Parbaharan, H. & Thompson, P. Therapeutic Morpholino-Substituted Compounds. WO 01/53266 A1, Thrombogenix, 2001.
128. Giranda, V. *et al*. Novel ATP-competitive Akt inhibitors slow the progression of tumors *in vivo*. *EORTC/AACR Molecular Therapeutics* Geneva 2005 Abstract # 246
129. Defeo-Jones, D. *et al*. Tumor cell sensitization to apoptotic stimuli by selective inhibition of specific Akt/PKB family members. *Mol. Cancer Ther*. **4**, 271–279 (2005).
130. Lindsley, C. W. *et al*. Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors. *Bioorg. Med. Chem. Lett.* **15**, 761–764 (2005).
131. Barnett, S. F. *et al*. Identification and characterization of pleckstrin-homology-domain-dependent and isoenzyme-specific Akt inhibitors. *Biochem. J.* **385**, 399–408 (2005).
132. Kondapaka, S. B., Singh, S. S., Dasmahapatra, G. P., Sausville, E. A. & Roy, K. K. Perifosine, a novel alkylphospholipid, inhibits protein kinase B activation. *Mol. Cancer Ther.* **2**, 1093–1103 (2003).
133. Van Ummersen, L. *et al*. A phase I trial of perifosine (NSC 639966) on a loading dose/maintenance dose schedule in patients with advanced cancer. *Clin. Cancer Res.* **10**, 7450–7456 (2004).
134. Eng, C. P., Sehgal, S. N. & Vezina, C. Activity of rapamycin (AY-**22**, 989) against transplanted tumors. *J. Antibiot. (Tokyo)* **37**, 1231–1237 (1984).
135. Dudkin, L. *et al*. Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. *Clin. Cancer Res.* **7**, 1758–1764 (2001).
136. Shi, Y. *et al*. Rapamycin enhances apoptosis and increases sensitivity to cisplatin *in vitro*. *Cancer Res.* **55**, 1982–1988 (1995).
137. deGraffenried, L. A. *et al*. Inhibition of mTOR activity restores tamoxifen response in breast cancer cells with aberrant Akt Activity. *Clin. Cancer Res*. **10**, 8059–8067 (2004).
138. Atkins, M. B. *et al*. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J. Clin. Oncol.* **22**, 909–918 (2004).
139. Chan, S. *et al*. First report: a phase 2 study of the safety and activity of CCI-779 for patients with locally advanced or metastatic breast cancer failing prior chemotherapy. *Proc. Am. Soc. Clin. Oncol.* **21**, abstract 175 (2002).
140. Galanis, E. *et al*. NCCTG phase II trial of CCI-779 in recurrent glioblastoma multiforme (GBM). *Proc. Am. Soc. Clin. Oncol.* **22**, 1503 (2004).
141. DeGraffenried, L. A. *et al*. Reduced PTEN expression in breast cancer cells confers susceptibility to inhibitors of the PI3 kinase/Akt pathway. *Ann. Oncol.* **15**, 1510–1516 (2004).
142. Mills, G. B., Lu, Y. & Kohn, E. C. Linking molecular therapeutics to molecular diagnostics: inhibition of the FRAP/RAFT/TOR component of the PI3K pathway preferentially blocks PTEN mutant cells *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **98**, 10031–10033 (2001).
143. Kau, T. R. *et al*. A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells. *Cancer Cell*. **4**, 463–476 (2003).
144. Kim, K. M & Lee, Y. J. Amiloride augments TRAIL-induced apoptotic death by inhibiting phosphorylation of kinases and phosphatases associated with the P13K-Akt pathway. *Oncogene.* **24**, 355–366 (2005).
145. Slamon, D. J. *et al*. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**, 783–792 (2001).
146. Paez, J. G. *et al*. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
147. Mukohara, T. *et al*. Differential effects of gefitinib and cetuximab on non-small-cell lung cancers bearing epidermal growth factor receptor mutations. *J. Natl. Cancer Inst*. **97**, 1185–1194 (2005).
148. Haas-Kogan, D. A. *et al*. Biomarkers to predict response to epidermal growth factor receptor inhibitors. *Cell Cycle*. **4**, (2005) [Epub ahead of print].
149. Giaccone, G. *et al*. A phase III clinical trial of ZD 1839 ('Iressa') in combination with gemcitabine and cisplatin in chemotherapy-naïve patients with advanced non-small cell lung cancer (INTACT-1). *Ann. Oncol.* **13**, 2 (2002).
150. Johnson, D. H. *et al*. ZD1839 ('Iressa') in combination with paclitaxel and carboplatin in chemotherapy-naïve patients with advanced non-small cell lung cancer (NSCLC): initial results from a phase III trial (INTACT-2). *Ann. Oncol.* **13**, 127 (2002).
151. Lynch, T. J. *et al*. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med*. **350**, 2129–2139 (2004).
152. Shepherd, F. A. *et al*. A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) following failure of 1st line or 2nd line chemotherapy. A National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) trial. *Proc. Am. Soc. Clin. Oncol.* **22**, 7022 (2004).
153. Moore, M. J. *et al*. Erlotinib improves survival when added to gemcitabine in patients with advanced pancreatic cancer. A phase III trial of the National Cancer Institute of Canada Clinical Trials Group [NCIC-CTG]. *ASCO Gastrointestinal Cancers Symposium* abstract 77 (2005).
154. Kohn, E. C. *et al*. Targeted Therapeutics in Breast Cancer: Challenges to Success. *Diseases of the Breast* (Editor Marc Lippman) In Press.
155. Kohn E. C. *et al*. Molecular Therapeutics: promise and challenges. *Semin Oncol* **31**, 39–53 (2004).
156. Drees, B. E., Mills, G. B., Rommel, C. & Prestwich G. D. Therapeutic potential of phosphoinositide 3-kinase inhibitors. *Expert Opinion on therapeutic patents* In Press.
157. Meric-Bernstam, F. & Mills, G. B. Mammalian target of rapamycin. *Semin Oncol* **31** (Suppl. 16), 10–17 (2004).
158. Debiec-Rychter, M. *et al*. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. *Gastroenterology* **128**, 270–279 (2005).
159. Bhalla, U. S., Ram, P. T. & Iyengar, R. MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network. *Science* **297**, 1018–1023 (2002).
160. Bhalla, U. S. & Iyengar, R. Emergent properties of networks of biological signaling pathways. *Science* **283**, 381–387 (1999).
161. Eker, S. *et al*. Pathway logic: symbolic analysis of biological signaling. *Pac Symp Biocomput* 400–412 (2002).
162. Ferrell Jr., J. E. & Machleder, E. M. The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes. *Science* **280**, 895–898 (1998).
163. Hoffman, A. *et al*. The IκB–NF–κB signaling module: temporal control and selective gene activation. *Science* **298**, 1241–1245 (2002).
164. Huang, C. Y. & Ferrell Jr., J. E. Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proc. Natl Acad. Sci. USA* **93**, 10078–10083 (1996).
165. Ma'ayan, A. *et al*. Formation of regulatory patterns during signal propagation in a Mammalian cellular network. *Science* **309**, 1078–1083 (2005).
166. Park, C. S., Schneider, I. C. & Haugh, J. M. Kinetic analysis of platelet-derived growth factor receptor/phosphoinositide 3-kinase/Akt signaling in fibroblasts. *J. Biol. Chem.* **278**, 37064–37072 (2003).
167. Sachs, K. *et al*. Causal protein-signaling networks derived from multiparameter single-cell data. *Science* **308**, 523–529 (2005).
168. Liu, X. *et al*. Rapamycin inhibits Akt-mediated oncogenic transformation and tumor growth. *Anticancer Res.* **24**, 2697–2704 (2004).
169. Wendel, H. G. *et al*. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* **428**, 332–337 (2004).
170. Podsypanina, K. *et al*. An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten$^{+/-}$ mice. *Proc. Natl Acad. Sci. USA* **98**, 10320–10325 (2001).
171. Neshat, M. S. *et al*. Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc. Natl Acad. Sci. USA* **98**, 10314–10319 (2001).

172. Pardanani, A. *et al*. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. *Blood* **104**, 3038–3045 (2004).
173. Druker, B. J. *et al*. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nature Med.* **2**, 561–566 (1996).
174. Samuels, Y. *et al*. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell*. **7**, 561–573 (2005).
175. Lu, Y., Zi, X. & Pollak, M. Molecular mechanisms underlying IGF-I-induced attenuation of the growth-inhibitory activity of trastuzumab (Herceptin) on SKBR3 breast cancer cells. *Int. J. Cancer* **108**, 334–341 (2004).
176. Ritter, C. A. *et al*. Mechanisms of resistance development against trastuzumab (Herceptin) in an *in vivo* breast cancer model. *Int. J. Clin. Pharmacol. Ther.* **42**, 642–643 (2004).
177. Thompson, J. E. & Thompson, C. B. Putting the rap on Akt. *J. Clin. Oncol.* **22**, 4217–4226 (2004).
Excellent review of AKT involvement in cancer cell metabolism.
178. Warburg, O., Posener, K. & Negelein, E. Uber den stoffwechsel der tumoren. *Biochem. Z.* **152**, 319–344 (1924).
179. Rathmell, J. C. *et al*. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. *Mol. Cell. Biol*. **23**, 7315–7328 (2003).
180. Bomanji, J. B., Costa, D. C. & Ell, P. J. Clinical role of positron emission tomography in oncology. *Lancet Oncol.* **2**, 157–164 (2001).
181. Samuelson, A. V. & Lowe, S. W. Selective induction of p53 and chemosensitivity in RB-deficient cells by E1A mutants unable to bind the RB-related proteins. *Proc. Natl Acad. Sci. USA* **94**, 12094–12099 (1997).
182. George, D. J. & Kaelin, W. G. Jr. The von Hippel–Lindau protein, vascular endothelial growth factor, and kidney cancer. *N. Engl. J. Med.* **349**, 419–421 (2003).
Excellent review of roles of vHL and VEGF in renal cell carcinoma.

Acknowledgements
The authors' research is supported by National Institutes of Health (NIH) grants to G.B.M. as well as by a Department Of Defence grant to P.T.R., and Aventis Pharmaceutical M.D. Anderson fellowship award to B.T.H., and a training fellowship from the Keck Centern Pharmacoinformatic Training Program at the Gulf Coast Consortia (NIH grant) to D.L.S.

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

Online links

DATABASES
The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
AKT1 | AKT2 | AKT3 | ATM | ATR | c-Kit | EGFR | HER2/*neu* | ILK | mTOR | p53 | p55γ | p85α | p85β | p110α | p110β | p110γ | p110δ | *PIK3CA* | PDGFR | Rb | vHL
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
CML | dermatofibrosarcoma protuberans | GIST | hypereosinophilic syndrome

FURTHER INFORMATION
Nucleic Acids Research Database List:
http://www3.oup.co.uk/nar/database/c/
The Sanger Institute Catalogues of Somatic Mutations in Cancer: http://www.sanger.ac.uk/genetics/CGP/cosmic/
The Signalling Gateway: http://www.signaling-gateway.org/
Access to this interactive links box is free online.