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Inhibiting PI3K as a therapeutic strategy against cancer

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Abstract Class I PI3K is composed of heterodimeric lipid kinases regulating essential cellular functions including proliferation, apoptosis and metabolism. Class I PI3K isoforms are commonly amplified in different cancer types and the PI3Kα catalytic subunit, PIK3CA, has been found mutated in a variable proportion of tumours of different origin. Furthermore, PI3K has been shown to mediate oncogenic signalling induced by several oncogenes such as HER2 or Ras. These facts suggest that PI3K might be a good target for anticancer drug discovery. Today, the rise of PI3K inhibitors and their first *in vivo* results have cleared much of the path for the development of PI3K inhibitors for anticancer therapy. Here we will review the PI3K pathway and the pharmacological results of PI3K inhibition.

Keywords PI3K · Cancer · Therapy · FOXO · Inhibitors

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The PI3K pathway

PTEN is a dual lipid and protein phosphatase. Its primary target is the lipid phosphatidylinositol-3,4,5-triphosphate (PIP3) [1], the product of phosphatidylinositol 3 kinase (PI3K). Loss of PTEN function, as well as PI3K activation, results in accumulation of PIP3, triggering the activation of its downstream effectors, PDK1, AKT/PKB and Rac1/Cdc42. The PI3K family is divided into 4 classes. Three of them have phosphorylated lipids as their main targets while class IV (mTOR, ATM, ATR and DNA-PK) has phosphorylated proteins. Class I, the most broadly studied, and the one we generally refer to in this review, is composed of heterodimers formed by a catalytic subunit (p110α, β, γ and δ) and a regulatory subunit. Class I can be subdivided into 2 subclasses: Ia, formed by the combination of p110α, β or δ and a regulatory subunit (p85, p65 or p55), and Ib, formed by p110γ and p101 regulatory subunit [2]. Activation of PI3K is induced by growth factors and insulin targeting the catalytic subunit to the membrane where it is in close proximity with its substrate, mainly PIP2. AKT contains a C-terminal pleckstrin homology (PH) domain, which binds the membrane-bound PIP3. AKT activity is regulated by PI3K activity in two steps. First, PIP3 recruits AKT to the cell membrane through AKT PH domain binding, permitting its activation by PDK1 [3]. PDK1 also contains a PH domain, which binds the membrane-bound PIP3, triggering its activation. Activated PDK1 phosphorylates AKT at T308, activating its ser/thr kinase activity. Once phosphorylated in T308, further activation occurs by phosphorylation at S473 by the complex mTORC2 or DNA-PK (Fig. 1). Activation of AKT results in the suppression of apoptosis induced by a number of stimuli including growth factor withdrawal, detachment of extracellular matrix, UV irradiation, cell cycle discordance and activation of FAS signalling [3–5]. Hyperactivated AKT has also been shown to promote cell

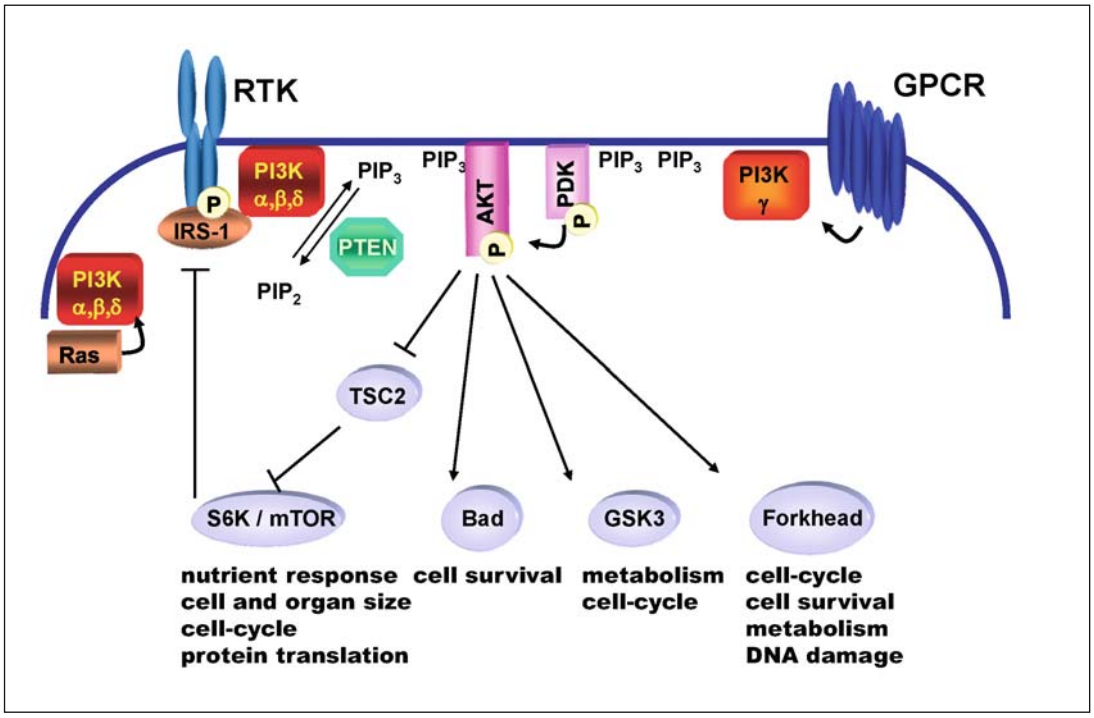

Fig. 1 General scheme of the PKB/AKT pathway

proliferation, cell growth and metabolism, resistance to hypoxia and migration [3, 6–10].

AKT is a serine/threonine kinase that enhances cell survival by blocking the function of proapoptotic proteins such as Bcl-2 family members [11, 12]. AKT also phosphorylates the FOXO family members FOXO, FOXO3a and FOXO4 [13] while they are in the nucleus, creating a binding site for 14-3-3 proteins, which trigger their export from the nucleus. Through this mechanism, AKT blocks FOXO-mediated transcription of target genes that promote apoptosis, cell-cycle arrest and metabolic processes. AKT also exerts some of its cell-survival effects through the modification of nutrient uptake and metabolism (reviewed in [5, 14]). AKT activation can stimulate proliferation through multiple downstream targets impinging on cell-cycle regulation. AKT phosphorylates the cyclin-dependent kinase inhibitors p21Cip1/WAF1 and p27Kip1, promoting its cytosolic localisation [15–18], and preventing its cell-cycle inhibitory effects. AKT activation can enhance the rate of glycolysis by promoting its ability to express glycolytic enzymes through HIFα [19, 20].

Deletion of AKT1 reversed the survival phenotype in PTEN null cells and abrogated its growth advantage [21]. Similarly, inactivation of AKT by dominant negative mutants inhibits the survival advantage provided by activated class I PI3K [22]. These and other results underline the essential role of AKT in the PTEN/PI3K pathway [23–27].

PI3K may control multiple pathways (PIP3 dependent or independent), besides the AKT pathway. PIP3-dependent functions, not related to AKT, might be PDK1 dependent as suggested by the hypomorphic PDK1 mice [28]. Reduced levels of PDK1 expression in PTEN(±) mice markedly protect these animals from developing a wide range of tumours. PDK1 has been shown to phosphorylate the critical residue in the activation loops of all AGC kinase family members including AKTs, SGKs, S6K, PKA, PKC, RSK and protein kinase N [2, 29]. Furthermore, other proteins might also be recruited and activated by an increase in PIP3 [4, 30]. The PH domain was the first phosphoinositide-binding domain identified. It is present in the largest number of proteins and is associated with the formation of signalling complexes on the plasma membrane. Recent studies identified other novel phosphoinositide-binding domains (Fab1p, YOTB, Vps27p, EEA1, Phox homology and epsin N-terminal homology (ENTH)), extending the functional versatility of the pathway (Fig. 2).

Therefore, targeting PI3K, the most proximal pathway component, is expected to present advantages over targeting more distal components such as AKT and mTOR. Inhibitors of PI3K diminish signalling to Rac as well as AKT, providing a broader inhibition of downstream signalling than distal inhibition.

The validation of PI3K as a drug target comes from different genetic sources. MEFs from p110$\alpha^{-/-}$ mice showed impaired tyrosine kinase signalling coupled to growth factors and are resistant for transformation with many tyrosine kinase oncogenes and Ras [31]. Although PIK3CB does not seem to be the target of mutations, it is activated in tumours with PTEN mutations. Indeed, tumours with PTEN mutations can be inhibited by selective targeting of PIK3CB [32, 33]. Thus, while targeting PI3Kα is expected to reverse the effects of PI3Kα mutations and PIK3CA

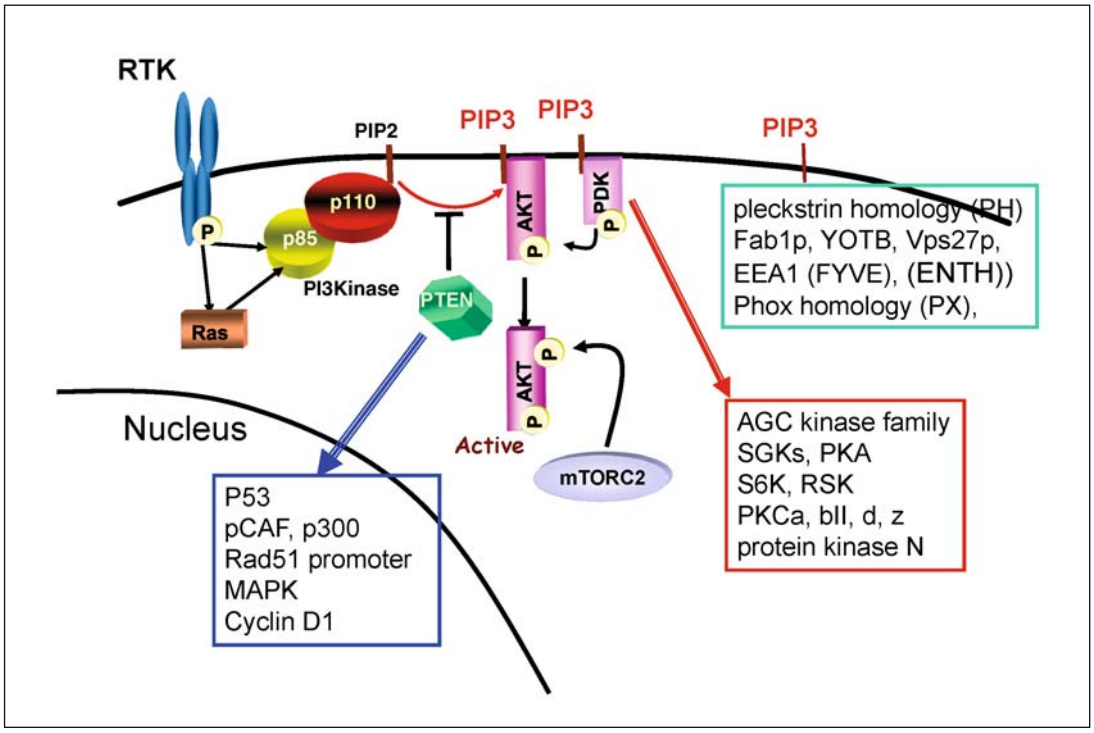

Fig. 2 General scheme of the alternative signalling to canonical AKT activation

amplification, targeting both PI3Kα and PI3Kβ might be required for optimal efficacy in PTEN mutant tumours. PI3Kδ and PI3Kγ may be particularly important in blood-derived neoplasms [33].

RAS

Activating point mutations in the genes encoding the Ras subfamily of small GTP-binding proteins contribute to the formation of a large proportion of human tumours [34]. The expression of this active version of Ras promotes tumour initiation by activating at least three different effectors: Raf, PI3K and RalGEFs [35–39]. Raf is a serine/threonine kinase that is localised to the plasma membrane from the cytoplasm and activated by GTP-Ras. Activated Raf proteins then initiate a MAP kinase (MAPK) signal transduction cascade leading to transformed morphologies, anchorage-independent growth and angiogenesis [38, 40]. Finally, the RalGEFs family of guanine exchange factors are activated via their recruitment to the plasma membrane by GTP-Ras [41]. In human cells it has been reported that the Ras effector pathways MAPK, RalGEF and PI3K are required to initiate tumour growth [42–45]. Conversely, activation of the PI3K/AKT pathway replaced Ras once tumours formed, although other effectors were still activated independently of Ras, presumably by factors provided upon the establishment of the tumour microenvironment. Thus, as tumorigenesis progresses the addiction of cancers to their initiating oncogene is reduced to, at least in the case of *Ras*, the PI3K/AKT pathway [45]. The genetic proof of the PI3K pathway relevance in *ras*-induced transformation was provided by Gupta and co-workers [46]. They generated mice with a mutant PI3KCA unable to interact with Ras. Cells from these mice show proliferative defects and selective disruption of signalling from growth factors to PI3K. They are highly resistant to endogenous Ras oncogene-induced tumorigenesis. The interaction of Ras with p110α is thus required *in vivo* for certain normal growth factor signalling and for Ras-driven tumour formation [46]. However, recent data from mouse models suggest that PI3K inhibitors efficiently block PI3K mutant-dependent lung tumours, but not tumours induced by oncogenic Ras [47]. However, combined PI3K and MEK inhibitor treatment efficiently blocks *ras*-dependent tumours. These data are consistent with those of Yu et al. [48], who found that PI3K inhibition resistance in cells is mainly determined by MEK pathway activation.

PI3K pathway in tumours

The PTEN/PI3K pathway is highly involved in cancer. PTEN activity is lost by mutations, deletions or promoter methylation silencing at high frequency in many primary and metastatic human cancers [10, 49]. Germline mutations of PTEN are found in Cowden, Bannayan-Riley-Ruvalcaba and Proteus-like syndromes, all familial cancer predisposi-

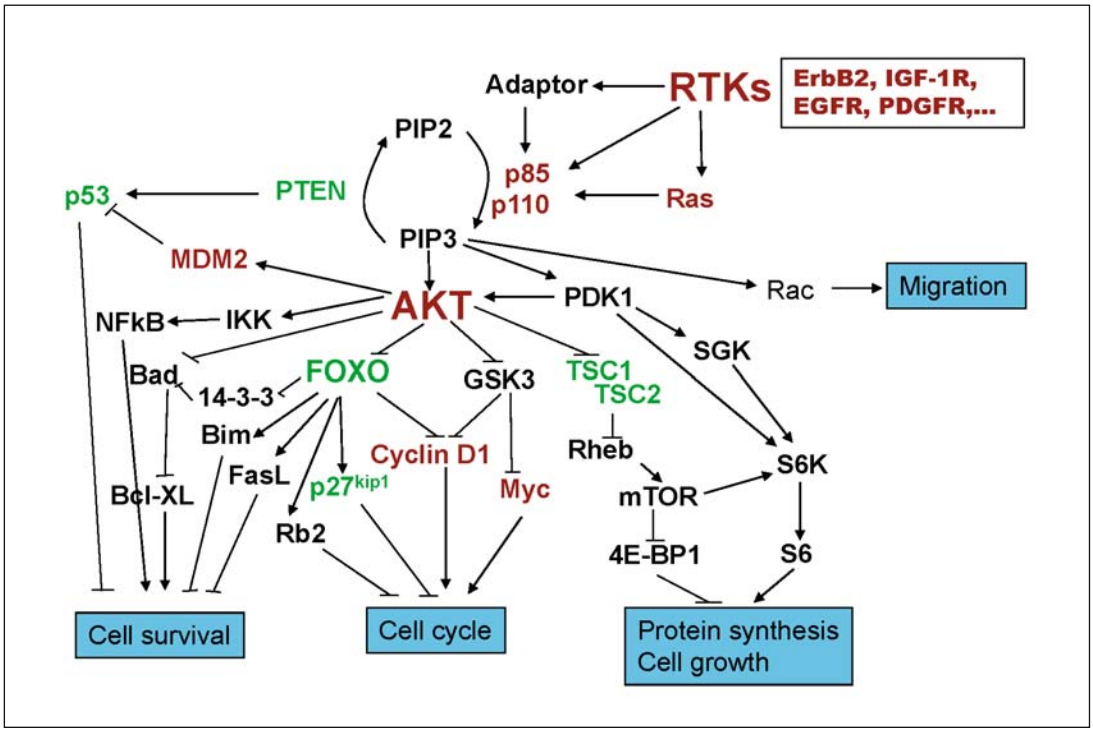

Fig. 3 General overview of PI3K pathway alterations found in human tumours

tion syndromes [50–53]. Recently, many activating mutations have been described in the PI3KCA gene (coding for the p110α catalytic subunit of PI3K) to be present in human tumours [49, 54]. The three most frequently observed PI3-kinase mutations: E542K, E545K and H1047R, showed enhanced catalytic activity [55], comparable to membrane-bound myr-p110. They are able to constitutively activate AKT and produce transcriptional activation. These enhanced biochemical capabilities translate to enhanced oncogenic activity of the PI3K mutants [56, 57]. Mouse models expressing activating mutations of PI3KCA found in human lung tumours (p110α–1047H) have been reported to produce lung adenocarcinomas. These tumours revert upon cessation of mutant PI3K expression [47]. We have targeted p110α to the cellular membrane activating PI3K in the mammary glands of transgenic mice [58, 59]. These mice are prone to spontaneous neoplasias [60, 61]. The oncogenic mutations have only been detected in the PI3KCA gene (p110α isoform), despite the observations that the activation, by membrane tagging, of all the class I PI3K isoforms have oncogenic potential [22, 56, 57].

Activation without mutations of PI3K are reported to occur in breast [62–64], ovarian [63, 65, 66], pancreatic [67], oesophageal [68], thyroid [69] and other cancers [49, 70].

This pathway is unique in that every major node is frequently mutated or amplified in a wide variety of solid tumours. Receptor tyrosine kinases upstream of PI3K, the p110α, AKT and the negative regulator PTEN are all frequently altered in cancer. Several other genes of the pathway can act as tumour suppressors such as TSC1, TSC2 or LKB1, which carry germline familial mutations, FOXO proteins and, probably, the phosphatases PHLPP and SHIP (Fig. 3).

However a closer analysis of some mutations in this pathway indicated there is no mutual exclusivity but in many cases coexisting mutations [71]. Coexistence of two or more PI3K pathway mutations in a single tumour would suggest differences in oncogenic mechanisms, given that there would be no selective advantage for cells bearing redundant mutations. Overall, PI3KCA mutations and PTEN loss coexist in breast, endometrial and colon cancers. Ras and PI3KCA mutations are mutually exclusive in endometrial cancers but coexist in colorectal cancers. This suggests that constitutively active RAS and PIK3CA may function synergistically in the colorectal epithelium to confer an important selective advantage [71]. In breast, HER2 is amplified in 30% of tumours [72] and appears to coexist with both PIK3CA mutations and PTEN loss. In fact, the coexistence of PTEN loss and HER2 amplification was critical to understanding trastuzumab resistance in HER2-positive breast cancers [73–75]. This suggests that PTEN loss and HER2 overexpression have redundant abilities to activate PI3K [71].

These data suggest that although PI3K may be a good target for therapeutic intervention, we have to be careful to understand the redundancy mechanisms before application to a specific subset of patients.

Therapeutic implications

The PI3K inhibitors LY294002 and wortmannin, both targeting the catalytic site of p110, have been largely used

as research tools to elucidate the value of PI3K as a therapeutic target. LY294002 and wortmannin have been found to be rather non-selective at active concentrations and present a toxic profile unsuitable for human testing [76–78]. However, some derivatives of these compounds have been generated and promoted to clinical testing. SF1126 is a water-soluble prodrug of LY294002 conjugated to a targeting peptide designed to increase solubility and bind to tumour vasculature [79]. This targeted prodrug enhances tumour delivery of the active inhibitor, improving antitumour efficacy and tolerability in xenograft models. LY294002 inhibits other kinases including mTOR, DNA-PK, PIM1, PLK1 and CK2, and induces oxidative stress in cancer cells independent of its PI3K inhibition. Phase I showed that SF1126 was well tolerated with no grade 3/4 drug-related toxicities reported. Thirty percent of patients showed stable disease for ≥8 weeks. Pharmacokinetics studies showed active hydrolysis of the product to LY294002 with evidence of target pathway inhibition and rapid clearing after termination [80].

Prodrugs of wortmannin have also been developed intended to extend its half-life in biological systems, and analogues created, which improve its pharmacologic properties [81]. PX-866 and PWT-458 are irreversible semisynthetic PEGylated derivatives of wortmannin selective for the PI3Kα, γ and δ isoforms [82, 83] that have higher therapeutic index in preclinical animal models compared to the parent compound. PX866 has also been included in phase I tests during 2008.

Many ATP derivative compounds have been found through screening campaigns and developed by MedChem with varying specificity for PI3K isoforms and other PIK family members, and their selectivity profiles have been determined through extensive profiling [77, 84]. Thus, many imidazopyridines, pyridopyrimidines, quinazolyne derivatives, thiazoles, azolepyrimidine derivatives and other chemotypes have been claimed as PI3K inhibitors [78]. Despite this, few compounds exhibit pharmacologic profiles suitable for advancement beyond preclinical testing [84]. These compounds are reported to be panClass I inhibitors with IC50 at nanomolar range [84]. Many of them are non-selective towards mTOR and DNA-PK [84]. All of them showed classic PI3K pathway inhibition in cells and *in vivo*, and are reported to possess antitumour activity in several xenograft models [2, 77, 85]. Overall, the activity of these ATP-competitive PI3K modulators translates well to *in vivo* models of human cancer. They are well tolerated and displayed disease stasis or even tumour regression when administered orally [86–88].

XL147 is a selective inhibitor of class I PI3K isoforms. In preclinical cancer models XL147 is cytostatic or cytoreductive as monotherapy and enhances the efficacy of targeted agents and chemotherapeutics [89, 90]. A phase 1 dose escalation study assessed the safety, pharmacokinetics, pharmacodynamics and efficacy of this compound in advanced solid tumours. XL147 was generally well tolerated, with the most common drug-related toxicity being skin rash. However, some grade 3 toxicities were observed [91]. A trend suggesting augmented food-induced changes in insulin was evident; however, glucose was minimally affected. XL147 reduced levels of phosphorylated PI3K pathway components in surrogated tissues and tumours in an exposure-dependent manner. In 2 patients dosed at the MTD, reductions of ≥70% in PI3K pathway signalling were observed in tumour tissue without compensatory upregulation of MEK/ERK phosphorylation. Prolonged stable disease for more than 6 months has been observed in 9 patients including 4 NSCLC [91].

XL765 is a potent and dual inhibitor of class I PI3K isoforms and mTOR. XL765 has shown dose-dependent target modulation and tumour growth inhibition or shrinkage in multiple human xenografts [92, 93]. Thirty-four patients have been dosed with XL765 at different regimens [94]. The most common drug-related adverse events were elevated liver enzymes, nausea and diarrhoea. XL765 augmented food-induced increases in plasma insulin, but not glucose, in an exposure-dependent fashion. Robust pharmacodynamic modulation of PI3K pathway signalling in surrogated tissues and tumours was evident following administration of XL765. Five patients had durable stable disease for more than 3 months [94].

GDC-0941 is a potent and selective oral inhibitor of class I PI3K with 3 nM IC50 for the p110α subunit *in vitro* and 28 nM IC50 in a cell-based pAKT assay and demonstrates broad activity in breast, ovarian, lung and prostate cancer models [84]. A phase I dose escalation study was performed in patients with solid tumours. GDC-0941 was generally well tolerated with no drug-related grade 3 or 4 toxicities observed. Grade 1 diarrhoea, nausea, dysgeusia, peripheral sensory neuropathy, dry mouth, thrombocytopenia and increased aspartate aminotransferase have been observed [95]. Preliminary data show decreased levels of pAKT in platelet-rich plasma correlating with GDC-0941 plasma concentrations. GDC-0941 effects on FDG-PET imaging are being assessed, with 1 patient with HER2+ metastatic breast cancer showing a reduction in FDG uptake and improvement of a chest wall lesion. GDC-0941 is generally well tolerated when administered at doses associated with inhibition of pAKT in surrogate tissues. Evidence of activity in tumour tissue has also been observed. Potential signs of anti-tumour activity have been observed in approximately 20% of patients [96].

BEZ235 and BTG226 are potent and dual PI3K-mTOR oral inhibitors with low nM IC50 for the PI3K and the mTOR activity *in vitro* and 15 nM IC50 in a cell-based pAKT assay. However, BEZ235 also shows strong DNA-PK inhibition. Both compounds entered phase I testing. A phase I/II, multicentre, open-label study of BEZ235, administered orally on a continuous daily dosing schedule in adult patients with advanced solid malignancies, followed by a safety expansion part and a phase II expansion part in breast tumours is currently ongoing. An effort will be made to enrich the trial population with Cowden syndrome patients with advanced solid malignancies [97].

CAL-101 (IC87114) is the only isoform-specific inhibitor reported to be in clinical trials for haematologic malignancies. CAL-101 specifically inhibits the PI3Kδ [98]. The PI3K p110δ isoform is highly expressed in cells of haematopoietic origin and plays a key role in B-cell maturation and function. CAL-101 is a potent inhibitor of PI3K p110δ (IC_{50}=65 nM) with 40–300-fold selectivity compared to other PI3K isoforms. *In vitro* studies of CAL-101 showed inhibition of pAKT expression and/or apoptotic effects against primary chronic lymphocytic leukaemia (CLL) and acute myeloid leukaemia (AML) cells and against a range of leukaemia and lymphoma cell lines. In an ongoing phase 1 dose escalation study in patients with relapsed/refractory CLL or select B-cell non-Hodgkin's lymphoma, CAL-101 was administered orally twice daily for 28 days per cycle. No treatment-related adverse events greater than grade 1 have been seen. Two of 6 patients attained partial response and 4 have stable disease. Partial responses were observed after 2 cycles of 50 mg in a patient with mantle cell lymphoma with 6 prior therapies, and after 1 cycle of 100 mg in a patient with follicular lymphoma with 6 prior therapies, including autologous stem cell transplant. Early results from a phase 1 study of the oral PI3K p110δ inhibitor CAL-101 show that it is well tolerated and has preliminary clinical activity in patients with B-cell malignancies [99].

Therapeutic combinations

The inhibitors of PI3K sensitise cancer cells to various types of conventional chemotherapy. LY294002 increases cytotoxicity induced by antimicrotubule agents such as taxanes and vinca alkaloids in glioma, ovarian cancer, oesophageal cancer, sarcoma and lung cancer cells *in vitro* and *in vivo* [100–103]. Wortmannin treatment sensitised cells to paclitaxel, cisplatin, gemcitabine or 5-fluorouracil [100, 104, 105], where potentiation of apoptosis caused by wortmannin was associated with inhibition of AKT activation. Potentiation of gemcitabine-induced apoptosis by PI3K inhibitors was associated with decreased Akt phosphorylation and increased levels of BAX in mitochondria [103]. Additionally, several studies have identified PI3K inhibitors as radiosensitisers and augmentation of radiation-induced cytotoxicity has been observed with suboptimal doses of wortmannin [106, 107] and LY294002 (Blanco and Carnero, Unpublished results). Similar results have been observed *in vitro* and *in vivo* between other PI3K inhibitors and many cytotoxic treatments used in oncologic therapy today [77].

Another possible approach is to combine inhibition of the PI3K/AKT/mTOR pathway with inhibition of a parallel prosurvival signalling pathway such as the MEK/ERK pathway [108]. This approach abrogates compensatory activation of other prosurvival pathways when the PI3K/AKT/mTOR pathway is inhibited. For example, combining an inhibitor of PI3K with an inhibitor of MEK causes a synergistic increase in apoptosis in both PTEN mutant and wild-type cells [109]. Cancer cell lines with mutant PTEN, which have high levels of AKT, are resistant to EGFR antagonists such as gefitinib and treatment with LY294002 restores gefitinib sensitivity [109]. Many different PI3K inhibitors can restore sensitivity to EGFR inhibitors. NSCLC cells transfected with gefitinib-sensitising EGFR mutations had increased levels of activated Akt and these cells were more sensitive than their wild-type counterparts not only to gefitinib, but also to LY294002 [110]. PX-866 was able to abolish gefitinib resistance in NSCLC xenografts [111].

Another potentially useful combination is proximal inhibition of erbB2, with distal inhibition of PI3K, AKT or mTOR. Inhibition of AKT phosphorylation is a requirement for the anti-proliferative effects of the erbB2 antagonist, trastuzumab, and trastuzumab-resistant cells exhibit sustained activation of the PI3K/AKT/mTOR pathway [73, 112]. In breast cancer cell lines and xenografts, PI3K inhibitors restored sensitivity to trastuzumab, concomitant with induction of apoptosis and inhibition of tumour growth [88, 113]. In addition to combining PI3K/AKT/mTOR inhibitors with agents that inhibit either the same or parallel prosurvival signalling pathways, PI3K/AKT/mTOR inhibitors have also been combined with targeted agents that defy easy categorisation such as imatinib and those that do not directly affect signalling pathways, such as histone deacetylase (HDAC) inhibitors [114, 115] and proteasome inhibitors [116]. Although the mechanisms behind the efficacy of these combinations are not completely understood, they represent potentially useful combinations for patients whose tumours do not respond to more conventional therapy regimens.

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