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PI3K inhibitors are finally coming of age

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Abstract

Overactive PI 3-kinase (PI3K) in cancer and immune-dysregulation has spurred extensive efforts to develop therapeutic PI3K inhibitors. Although progress has been hampered by issues such as poor drug tolerance and drug resistance, several PI3K inhibitors have now received regulatory approval – the PI3Kα isoform-selective inhibitor alpelisib for the treatment of breast cancer, and inhibitors mainly aimed at the leukocyte-enriched PI3Kδ in B-cell malignancies. In addition to targeting cancer-cell intrinsic PI3K activity, emerging evidence highlights the potential of PI3K inhibitors in cancer immunotherapy. This review summarises key discoveries aiding the clinical translation of PI3Kα and PI3Kδ inhibitors, highlighting lessons learned and future opportunities.

TOC

PI3K signalling is one of the most frequently aberrantly-activated pathways in cancer. However, the development of therapeutic PI3K pathway inhibitors has faced challenges including poor drug tolerance and drug resistance. Here, Vanhaesebroeck et al. review efforts to understand and therapeutically exploit the biology of PI3Kα and PI3Kδ — the key targets of currently approved PI3K inhibitors, highlighting lessons learned and future opportunities.

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Keywords

PI3Kα; PI3Kδ; *PIK3CA* ; *PIK3CD* ; breast cancer; lymphoma; immunity; inflammation; cancer; immunotherapy; drug development

Introduction

Class I PI3Ks signal downstream of tyrosine kinases, G protein-coupled receptors (GPCRs) and GTPases such as Ras, Rac and Cdc42, to regulate a range of cellular activities, including metabolism, proliferation and migration (Figure 1)[1,2]. PI3K signalling is one of the most frequently aberrantly-activated pathways in cancer, and early studies showed that the pan-PI3K inhibitors LY294002 and wortmannin could revert cancer cell resistance to a broad range of therapies, including chemotherapy, radiation and targeted therapies[3]. Some PI3K family members are also involved in inflammation and auto-immunity[4-7].

Class I PI3Ks consist of a regulatory subunit in complex with a p110 catalytic subunit (p110α, β, γ and δ). Below, these heterodimeric complexes will be referred to as PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ, with p110α, p110β, p110γ and p110δ indicating the catalytic subunits themselves. Whereas p110α and p110β show a broad tissue distribution, p110γ and p110δ are highly enriched in all leukocyte subtypes, with emerging data of low but functionally-relevant levels of p110δ in non-leukocytes.

Class I PI3Ks generate phosphatidylinositol-(3,4,5)-trisphosphate (PtdIns(3,4,5)P_3, or PIP_3) which can be converted to PtdIns(3,4)P_2 by 5-phosphatases such as SHIP1 and SHIP2 (Figure 1). PIP_3 and PtdIns(3,4)P_2 interact with 3-phosphoinositide-binding pleckstrin homology (PH) domains found in diverse proteins, including protein kinases (such as AKT/PKB, BTK), adaptor proteins and regulators of GTPases, to regulate their activities. The tumour suppressor phosphatase and tensin homolog (PTEN), which is frequently somatically inactivated in cancer, dampens class I PI3K signalling (Figure 1).

Given its key role in cancer and immunity, the PI3K pathway has been the focus of extensive drug development efforts in the past two decades. In 2014, the PI3Kδ inhibitor idelalisib (Zydelig/CAL-101/GS-1101; Gilead Sciences) became the first PI3K inhibitor to be approved, for use in specific B-cell malignancies. This was followed by the approval in 2017 of the pan-class I PI3K inhibitor copanlisib (Aliqopa/BAY 80-6946; Bayer) and in 2018 of the dual PI3Kδ/γ inhibitor duvelisib (Copiktra/IPI-145/INK1197; Verastem, now Secura Bio) for the same indications (Table 1). Umbralisib (TGR-1202; TG Therapeutics)[8] has recently received fast track status in CLL in combination with the anti-CD20 antibody ublituximab, as well as conditional FDA approval in follicular lymphoma and marginal zone lymphoma[9] (Table 1). In 2019, the PI3Kα inhibitor alpelisib (Piqray/NVP-BYL719; Novartis) was approved for the treatment of advanced breast cancer, in combination with the oestrogen receptor (ER) down-regulator fulvestrant[10].

Although these approvals have validated the pathway as a viable drug target, the development of PI3K pathway inhibitors has proven challenging, with progress hampered by poor drug tolerance, intrinsic and acquired drug resistance and signalling feedback loops

that neutralize PI3K inhibition[11-14]. The lack of clinical benefit and poor tolerability of pan-class I PI3K and dual PI3Kα/δ inhibitors has halted further clinical development of these compounds. Nevertheless, the development of isoform-selective PI3K inhibitors and increased clinical experience with PI3K inhibitors are now heralding a more productive phase in PI3K drug development.

Here, we overview efforts to understand and therapeutically exploit the biology of PI3Kα and PI3Kδ — the key targets of currently approved PI3K inhibitors —and the lessons learnt in their development, to realize the potential of this drug class. Data on PI3Kβ and PI3Kγ are also mentioned where relevant. The current landscape of PI3K inhibitors and the general principles of isoform-selective PI3K inhibitor development are summarized. Reflecting the differing roles of PI3Kα and PI3Kδ, their respective biologies, the clinical experience targeting these PI3Ks and emerging opportunities will be discussed, closing with a perspective on the future of the field overall.

The PI3K inhibitor landscape

The class I PI3K p110 catalytic subunits consist of an N-terminal adaptor-binding domain, a Ras binding domain, a membrane binding C2 domain, a helical domain and a C-terminal catalytic domain which is divided into N- and C-terminal sections and separated by the hinge, where ATP is bound (Figure 1; insert)[15,16]. The regulatory subunits bind to and maintain the p110 subunits in an inactive form until the PI3Ks become activated by engagement of their regulatory subunits with upstream signalling inputs.

Most PI3K inhibitors are ATP-competitive. The ATP-binding pocket is in a cleft between the two lobes of the kinase domain, with a hinge valine residue at the end of the cleft (shown in p110γ in Figure 2a). This valine is conserved in all class I PI3K isoforms and forms an H-bond with the purine ring of ATP. Accordingly, all ATP-competitive PI3K inhibitors identified to date accept an H-bond from this valine residue.

A series of non-ATP competitive PI3Kδ inhibitors have also been identified, illustrated by the PI3Kδ inhibitor IOA-244 (iOnctura; Supplementary Figure 1a)[17], but the structural details of the binding mode of these molecules have not been disclosed.

Non-isoform selective inhibitors

The native shape of PI3K enzymes is taken to be that observed by crystallography for ATP-bound p110γ (PDB:1E8X)[18] or the very similar apo forms observed for p110γ (PDB:1E8Y)[18], p110δ (PDB: 2WXR)[19] and PI3Kα (in complex with a partial p85α fragment, PBD:2RD0)[20].

Early PI3K inhibitors exhibited similar activity against all class I PI3K isoforms, for example buparlisib[21,22] (Supplementary Figure 1b), with copanlisib (Figure 2b) representing an optimised development of these chemotypes[23]. The conformation of p110γ bound to copanlisib (PDB: 5G2N)[23] is almost unchanged from p110γ bound to ATP: copanlisib binds in the ATP binding site with the nitrogen atom of the imidazolidine making the obligatory

H-bond with the NH group of the Val882 hinge residue, while its flat core heterocycle fits neatly between the hydrophobic faces of the cleft (Figure 2b).

Obtaining selectivity beyond the conserved ATP pocket

Although flat inhibitors are typically non-selective, it is possible to obtain selectivity from such compounds by making larger molecules whose binding extends beyond the conserved region of the ATP-binding pocket. Thus, alpelisib gains selectivity and potency for PI3Kα by addition of functionality at both termini of the molecule that make specific interactions[24] (Figure 2c). Taselisib (GDC0032) (Figure 2d) makes use of a similar carboxamide to alpelisib, but is also capable of binding to PI3Kδ with high affinity[25]. Inavolisib (GDC-0077; Figure 2e), a further development of the taselisib structure, is significantly more selective and inhibits only PI3Kα (Table 2). Inavolisib makes more precise interactions in the affinity pocket of p110α along with the PI3Kα favouring carboxamide to give excellent PI3K isoform selectivity[25].

Other differences at the edge of the ATP binding pocket of the PI3K isoforms have also been exploited to identify isoform-selective PI3K inhibitors. In the case of p110δ, differences in the residue corresponding to Thr750 in p110δ (Arg770, Lys777, Lys802 in p110α, β and γ, respectively) mean that p110δ is able to accommodate large groups that can occupy the exposed face of Trp760 (the so-called Trp shelf) in p110δ[26]. Most notably, the exquisitely PI3Kδ-selective inhibitor nemiralisib (GSK2269557, 5AE8)[27] (Figure 3a) puts an isopropyl group in this position whilst the less selective leniolisib/CDZ173 (Figure 3b) has a propionamide over Trp 760.

In p110γ, Ala885 corresponds to serine in the other class I PI3K isoforms, whilst Gly829 corresponds to glutamine in p110α; these differences were exploited in the design of moderately PI3Kγ-selective compounds[28]. Further modifications retained PI3Kγ-selectivity making use only of the difference at Ala885[29].

Inhibitors forming a specificity pocket

The most widely used selectivity driver in PI3Kδ is the formation of a pocket (“specificity pocket”) by inhibitors inducing the movement of a methionine (Met752 in p110δ) relative to a tryptophan (Trp760 in p110δ). Idelalisib[30], duvelisib[31], seletalisb[32] and umbralisib[8] all use this pocket (Figure 4a-e).

The highly selective PI3Kδ inhibitor parsaclisib (INCB50465; Incyte; Figure 4f)[33] appears to be an optimised so-called ‘propeller structure’. Although a crystal structure of parsaclisib bound to PI3K has not been published, docking studies suggest that the carbonyl group of a pendant lactam accepts two H-bonds Thr750 and Lys708 that serve to anchor the molecule in the enzyme. Thus, as with later generation PI3Kα inhibitors, building in additional H-bonds with non-conserved residues confers increased PI3K isoform selectivity.

Other drivers for PI3K isoform selectivity with this PI3K pocket are, however, extremely subtle, since not only the original PI3Kδ-selective inhibitors make use of this pocket[19] but also PI3Kγ/δ inhibitors[34], PI3Kβ/δ[35] and PI3Kβ-favouring[36] inhibitors. Although no structural information has been disclosed, it is likely that, based on the chemical core, even

the highly PI3Kγ-selective propeller-shaped inhibitor eganelisib/IPI-549 (Supplementary Figure 1c) makes use of the same pocket[37].

Thus, optimisation of the shape and functionality of inhibitor structures enables multiple different PI3K isoform selectivity patterns to be obtained from a single PI3K pocket.

Other PI3K inhibitors

Other notable inhibitors include a PI3Kα inhibitor chemotype that was identifed through DNA-encoded library screening, which is very different to any previously identified PI3K inhibitor and makes key interactions through a carboxylate group with a non-conserved Arg770 in the P-loop and the non-conserved Gln859 in the C-terminal lobe (4YKN)[38]. Extending even further from the ATP binding site, careful design of an acrylamide substituted inhibitor (3ZIM) generated compounds that form a covalent bond with Cys862[39]. Whether such covalent inhibitors have additional advantages or liabilities remains to be determined.

In addition to eganelisib/IPI-549, an alternative means of obtaining selectivity for PI3Kγ has been discovered in a series of inhibitors that bind to the inactive form of the kinase but then induce a conformational change in p110γ, leading to a rearrangement of the enzyme to an active-like conformation[40]. This rearrangement is due to a substituent of the inhibitor extending deep into the affinity pocket and occurs in two stages, the first causing a movement of the conserved ATP-binding DFG motif in the kinase activation loop and the second a larger reorganisation of the kα12 helix and the kα4-kα5 loop. This process is thought to be easiest in p110γ and accounts for the very high PI3K isoform selectivity observed[41].

PI3Kβ-selective inhibitors have been harder to find[42], but BL140 represents one of the most selective tool compounds reported, with 150-fold and 430-fold selectivity against PI3Kα and PI3Kδ, respectively (no data given for PI3Kγ)[43] (Supplementary Figure 1d). For PI3Kβ inhibitors that have entered clinical development, for example SAR260301, GSK2636771 and AZD8186 (Supplementary Figure 1e-g), the selectivity, where reported, has been lower.

In vivo PI3K isoform-selectivity

Whilst PI3K isoform selectivity in cells and tissues is difficult to predict based on *in vitro* biochemical data, it unlikely that most approved compounds inhibit only a single PI3K isoform in the clinical setting.

Thus, idelalisib has only 36-fold selectivity for PI3Kδ over PI3Kγ[30]. Duvelisib is closely related to idelalisib (differing only by 4 atoms) and is more potent, but less isoform-selective and is described as a dual PI3Kγδ inhibitor, with 11- and 34-fold selectivity for PI3Kδ over PI3Kγ and PI3Kβ, respectively[31]. It is therefore likely that idelalisib as well as duvelisib inhibit both PI3Kγ and δ in patients, at least for significant periods during drug dosing. In contrast, the second-generation PI3Kδ inhibitor umbralisib has much greater isoform selectivity (PI3Kα, PI3Kβ: >1000 fold, PI3Kγ: 225 fold) and this may contribute to a better safety profile (discussed further below).

Copanlisib is closer to a pan-class I PI3K inhibitor, with similar levels of activity against PI3Kα and PI3Kδ, and about 7- or 13-fold more selective for PI3Kα over PI3Kβ or PI3Kγ, respectively[23].

While alpelisib exhibits the highest selectivity for PI3Kα, with 50-, 63- and 260-fold selectivity over PI3Kγ, PI3Kδ and PI3Kβ, respectively[24,44], it is likely that some inhibition of PI3Kγ and PI3Kδ will occur, at least for some patients, during dosing of this drug.

PI3Kα: from biology to approved drugs

Physiological roles of PI3Kα

At the cellular level, a key function of PI3Kα is to convert growth factor stimulation into activation of anabolic processes (glucose uptake, glycolysis, nucleotide production, protein and lipid synthesis) and concomitant inhibition of catabolic processes (including autophagy). A key effector of PI3Kα in this response is AKT/PKB, a serine/threonine kinase with a myriad of substrates and pleiotropic functions. AKT is critical for transduction of growth factor stimulation through activation of the master regulator for cell growth, the mTORC1 protein kinase complex (which also receives class I PI3K-independent input from amino acids and glucose). Combined, AKT and mTORC1 set the stage for enhanced energy generation and biosynthetic activity, key requisites for cell proliferation and survival. The ensuing metabolic shift is associated with increased levels of several metabolites, including acetyl-CoA, that serve as substrates of chromatin-modifying enzymes[45,46]. This endows the PI3K pathway with the ability to elicit widespread transcriptional changes beyond those attributed to the action of individual signalling effectors.

The role of PI3Kα in the regulation of the cell cytoskeleton, for example through the regulation of GEFs and GAPs for small GTPases[47] or actin-binding proteins such as gelsolin[48], remains to be fully explored. Such an effect of PI3Kα has been implicated in glycolysis whereby PI3K activates Rac, resulting in actin cytoskeleton remodelling, allowing the release into the cytosol of actin-bound aldolase, a rate-limiting enzyme of glycolysis[49].

The generation of mice in which endogenous PI3Kα was rendered inactive[50] and the use of isoform-selective PI3K inhibitors[51], positioned PI3Kα as the main insulin signalling PI3K isoform. Partial PI3Kα inactivation in mice leads to blunted insulin signalling, hyperinsulinaemia and glucose intolerance[50], later found to be the main on-target adverse clinical effects of any inhibitor with activity against PI3Kα[52].

PIK3CA in cancer

Genetic PI3Kα activation in cancer—*PIK3CA* is the most frequently mutated kinase in solid tumours (14% mutated across all cancers, but rarely in haematological malignancies[53]). Interestingly, normal endometrial epithelium also frequently carries oncogenic *PIK3CA* and *PIK3R1* mutations, the burden of which increases with age and decreases with parity[54].

Oncogenic mutations are present across *PIK3CA*,apart from the Ras-binding domain, but highly enriched for ‘hotspot’ mutations in the helical (E542K, E545K) and kinase

(H1047R) domains[55], which also have the strongest biological impact in experimental cell model systems compared to other *PIK3CA* mutations[56]. It is likely that different *PIK3CA* mutations have distinct biological outputs, as reported in a glioblastoma mouse model[57], but this remains to be investigated in detail. Two activating mutations frequently co-occur in cis on the same *PIK3CA* allele[58-60], the expression of which may render such cells more sensitive to PI3Kα inhibitors compared with cells with single-hotspot *PIK3CA* mutations[58].

Oncogenic mutations in *PIK3CA* mimic and enhance dynamic events in the natural activation process of the auto-inhibited p85-p110 heterodimer[15,61,62]. Such processes can also be achieved by mutations in the p85 genes, most commonly in *PIK3R1*[63-65]. *PIK3R1* mutations, common in cancers such as endometrial carcinoma[66], can also activate p110β and p110δ in addition to p110α[65].

In mouse models, heterozygous *PIK3CA* mutation alone is a poor inducer of cancer, but is effective in combination with other oncogenic lesions, including mutated *BRaf* or *KRas* or loss of tumour suppressor genes such as *Pten*, *Tp53* or *Apc*[67]. By contrast, transgenic *over*-expression of oncogenic *PIK3CA* can induce cancer on its own, correlating with the emerging evidence for the dose-dependency of genetic PI3K pathway activation in cancer[68].

In some cancers (including breast[69] and colon[70]), *PIK3CA* mutation can be an early, clonal event and thus present in all cells. In other cancers, *PIK3CA* mutation occurs at later stages of tumour evolution and is thus subclonal and not present in all tumour cells[71]. The latter has obvious therapeutic implications if PI3K inhibitors would only effectively target *PIK3CA* mutant cells. A substantial subset of human cancers has multiple copies of mutant *PIK3CA*[68], in line with findings that cancers often acquire multiple oncogenic hits within the PI3K pathway[72,73]. Positive selection for oncogenic mutant allele imbalances is frequent in cancer and has also been documented for Ras and other oncogenes[74,75]. Evidence for a sharp, dose-dependent biological impact of the $PIK3CA^{H1047R}$ hot-spot mutation was documented in human induced pluripotent stem cell models, where $PIK3CA^{H1047R}$ heterozygosity led to negligible biological impact compared to homozygous expression[68].

Wild-type *PIK3CA* is frequently amplified in some cancers[53] such as endometrial[76] and lung squamous carcinoma[77], as part of an amplification of the 3q genomic locus. That *PIK3CA* amplification may have functional relevance is indicated by its ability to predict *in vitro* sensitivity to alpelisib in a cancer cell line panel[44]. However, in contrast to expression of mutant *PIK3CA*[78,79], overexpression of wild-type p110α appears to have minor, if any, effects on PI3K pathway stimulation as assessed by phosphorylation of AKT/PKB, both under basal and growth-factor-stimulated conditions[80,81]. Overexpression of wild-type human *PIK3CA* does also not show transforming capacity in a chicken fibroblast assay, in contrast to oncogenically-mutated or membrane-targeted versions of human wild-type *PIK3CA*[79]. It is challenging, however, to overexpress p110α protein in cells, most likely because the limiting availability of p85 that is needed to stabilize the labile p110α protein.

Pleiotropic impact of PIK3CA mutation—In isogenic cancer cell lines, derived by disruption of the wild-type or mutant allele of *PIK3CA*[78,82], *PIK3CA* mutation has multiple impacts: reduced growth factor dependence yet little effect on cell proliferation under

nutrient-rich conditions[78], increased *in vitro* cell migration and invasion[78,83] and reduced sensitivity to starvation-induced apoptosis[78] (Figure 5a). At the cellular level, the impact of activating *PIK3CA* mutations is context-dependent, and can range from no effect to enhancement of cell proliferation to cell senescence[82,84-86] or even cell death[87] (reviewed in Ref.[88]). Interestingly, in cells with functional p53, *PIK3CA* mutation leads to activation of p53-dependent growth suppression[82].

Accumulating evidence suggests that oncogenic PI3Kα activation supports the emergence of stem cell-like properties[89]. Activating *PIK3CA* mutations also promote invasive properties and epithelial-to-mesenchymal transition (EMT)[80,83,90], which is strongly associated with induction of stemness, phenotypic plasticity and, ultimately, resistance to targeted therapy[91-93]. EMT may also facilitate tumour invasion and metastasis[78,83]. The mechanism(s) driving mutant *PIK3CA*-dependent cellular plasticity and EMT remain poorly understood, with a possible involvement of reciprocal dependency between the PI3K and TGFβ signalling pathways[86,94-97].

Genetic *PIK3CA* activation may also induce and/or allow cells to tolerate chromosomal instability[98], potentially facilitating and/or driving tumour evolution[99].

There is also increasing evidence for paracrine effects of oncogenic PI3Kα activation. Transcriptional profiling of a *PIK3CA*-mutant derivative of the MCF10A breast cell line indicated the expression of PI3K-driven, NF-κB–dependent target-genes enriched in cytokines, chemokines or secreted proteins[81]. Upon overexpression of the Human Epidermal growth factor Receptor 2 (HER2) in these cells, *PIK3CA* mutation induced a complex secretome that promotes stem cell enrichment, angiogenesis, EMT, altered immune surveillance and vulnerability towards HSP90 inhibition[86]. *PIK3CA* mutation in breast cancer cell lines is associated with a lipogenic subtype that depends predominantly on mTORC2 activation, with intracellular and secreted arachidonic acid and its metabolites fueling cancer-cell intrinsic proliferation but also that of surrounding *PIK3CA* WT cells[100].

PIK3CA mutation in cancer cells might also create an immunosuppressive stromal environment by induction of high glycolysis in cancer cells, leading to a high demand for glucose[101,102] and subsequent depletion of metabolic fuels in the stroma,thus contributing to immune suppression[103].

In a mouse model of glioblastoma, $PIK3CA^{C420R}$-mutant glioblastoma cells affect neighbouring neurons through the secretion of glypican family proteins that can increase synaptic activity in neurons[57], a phenomenon possibly related to the seizures often observed in glioblastoma. Likewise, the secretion of interleukin-6 by $PIK3CA^{H1047R}$ breast epithelial cells has been implicated in increasing permeability and structural disorganization of the neighbouring endothelium[104].

Activating mutations in the PI3K/AKT/MTOR pathway commonly cause paediatric intractable epilepsy, which in mouse models has been shown to be driven by non-synaptic, neuron-intrinsic properties, involving calcium and potassium channels, and to be suppressible by acute treatment with PI3K pathway inhibitors[105]. PI3K pathway inhibitors therefore potentially represent novel anti-seizure therapeutics in this condition.

In conclusion, *PIK3CA* mutation has a diverse biological impact on cells beyond stimulation of cell proliferation.

PI3K inhibition in cancer

In vitro cytostatic effects—A common misconception is that PI3K inhibition leads to cancer cell death. However, this is not typically the case, at least upon *continuous* drug exposure of cancer cell lines *in vitro* where PI3K inhibitors in sensitive cell lines most often lead to inhibition of cell proliferation rather than cell death[106-109]. This arrest may be akin to a 'dormant' state, as observed upon inactivation of the AGE-1 class I PI3K catalytic subunit in *C. elegans*[110]. Cell-based studies with PI3K inhibitors have mainly used assays that measure protein content or metabolic activity of cells (for example, sulforhodamine B, MTT/MTS or CellTitre-Glo assays) which are not *bona fide* readouts of cell death. Published evidence for the induction of cell death by PI3K inhibitors mostly derives from PARP or caspase cleavage as measured by western blotting, however the levels induced are most often low and may only represent a small fraction of the total cell population (see for example Ref.[111]). However, significant cancer-cell cytotoxicity has been reported with some PI3K inhibitors, such as upon treatment with the pan-PI3K inhibitor copanlisib[112] or upon *intermittent* dosing with the dual PI3Kα/δ inhibitor AZD8835[113].

It should also be noted that PI3K inhibitor studies have mainly used conditions that do not reflect those in a tumour, with cells seeded at low density in 2D in nutrient-replete conditions under normoxia. Tumours in humans also have far longer doubling times than those used in xenograft models[114,115].

Sensitivity to PI3Ka inhibitors—The presence of activating *PIK3CA* mutations is the clearest positive predictor of *in vitro* sensitivity of cancer cell lines to the anti-proliferative effect of alpelisib[44] or the dual PI3Kα/δ inhibitors AZD8835[113] and taselisib[116]. This correlation is not absolute but has held up well in breast cancer patients treated with alpelisib[10]. *PIK3CA* amplification is also an independent predictor of *in vitro* sensitivity to alpelisib[44].

At the cellular level, intrinsic and acquired resistance to PI3K inhibitors is very common[14] (BOX1). At the organismal level, the anti-proliferative effects of PI3K inhibitors are neutralised by compensation for the metabolic impact of PI3Kα inhibition. Indeed, PI3Kα inhibition in preclinical mouse models leads to reduced glucose uptake in insulin-responsive tissues such as adipose tissue and muscle, which results in hyperglycaemia and a compensatory insulin release from the pancreas, thereby dampening the effect of PI3K inhibition[12].

Clinical development of PI3Kα inhibitors

The approved PI3Ka inhibitor alpelisib—The main rationale for the use of PI3K inhibitors in oncology has been to target cancer-cell-intrinsic PI3K activity. Given the cytostatic effect of PI3K inhibitors on tumour cells[106-109], their cell-intrinsic impact as single agents may primarily result in tumour stabilisation, rather than regression. Drug combination approaches have therefore been explored.

The most compelling of these approaches is in hormone-responsive breast cancer, a prime example of context/tissue-specific effects of PI3K inhibition[88]. Indeed, preclinical data have shown that PI3K pathway inhibition often mediates resistance to anti-oestrogen therapies[117,118], observations in line with clinical data that demonstrated improved progression-free survival (PFS) in ER-positive breast cancer by combination treatment with the mTORC1 inhibitor everolimus and an aromatase inhibitor[119] (reviewed in Ref.[120]). Likewise, PI3Kα-selective inhibitors enhance oestrogen pathway activity in breast cancer and increase their dependence on this hormone, through modulation of ER transcription in conjunction with epigenetic effects (Figure 5b)[121].

Other targeted therapies also enhance the efficacy of endocrine therapy, including inhibitors of AKT[122], mTOR or CDK4/6 (reviewed in Ref.[123]), some of which are likely to be contenders of PI3Kα inhibitors in this clinical setting.

The PI3Kα selectivity of alpelisib (Table 1)[24] and its pharmacokinetics enabled successful trials in breast cancer, leading to the drug's FDA approval in 2019[124]. The SOLAR-1 trial (NCT02437318)[10] compared the effect of the ER antagonist fulvestrant with or without alpelisib, finding that combination treatment prolongs PFS among patients with *PIK3CA*-mutated, ER-positive, HER2 receptor-negative ($HR^+/HER2^-$) advanced breast cancer who had received prior endocrine therapy. Importantly, alpelisib did not affect PFS in patients without *PIK3CA* mutation.

At the mature analysis of the SOLAR-1 trial, the median overall survival was 39.3 months with alpelisib plus fulvestrant compared with 31.4 months with placebo plus fulvestrant[125]. The most frequent side-effects with alpelisib are hyperglycaemia, rash and diarrhoea[126,127], which are manageable and reversible.

The SOLAR-1 trial did not have a PI3Kα inhibitor-only arm and a key question is how much of the observed clinical response is due to direct anti-cancer effects of PI3Kα inhibition and how much derives from the restoration of cellular sensitivity to anti-oestrogen therapy by PI3Kα inhibition.

Other PI3Ka-selective inhibitors in clinical trials—Taselisib (Genentech), a highly-potent dual PI3Kα/δ inhibitor[25,128] progressed to Phase III studies in breast cancer (Table 2). However, development was discontinued due to modest clinical benefit and considerable adverse side effects, with 51.4% of patients stopping treatment due to gastrointestinal toxicities[129], possibly due to PI3Kδ inhibition.

Using the same core and key amide as taselisib, inavolisib (Genentech; Figure 2e) was generated, with enhanced PI3Kα isoform selectivity, and is currently being trialled in breast cancer in combination with a range of endocrine and targeted therapies (NCT03006172; NCT04802759) and other solid tumours (NCT04632992). A study currently under peer review has reported taselisib and inavolisib to lead to degradation of the E545K and H1047R mutant p110α proteins in cells, an effect not seen with other PI3Kα-selective inhibitors such as alpelisib[130]. This degradation of mutant p110α appears to block feedback-induced PI3K pathway reactivation in cells, resulting in enhanced potency of taselisib and inavolisib,

compared to other PI3K inhibitors in cancer cell and xenograft studies[130]. This inhibitor-induced p110α-mutant proteasome-mediated degradation is dependent on receptor tyrosine kinase activity in *PIK3CA*-mutant cells, and is especially prominent in cells with HER2 amplification[130]. HER2-targeted therapy is the standard of care for HER2-amplified breast tumors, with HER2-positive, *PIK3CA* mutant breast cancer known to be less responsive to HER2-targeted therapy. Based on these data, Genentech is now planning a clinical trial to test inavolisib in combination with HER2 antibodies.

Serabelisib (INK-1117/MLN-1117/TAK-117/ART-001; Figure 2f) is another selective PI3Kα inhibitor. Though not particularly potent against PI3Kα, requiring substantial doses, serabelisib has excellent isoform selectivity[131] and favourable pharmacokinetics[132]. The structural basis for the PI3K isoform selectivity of serabelisib has not been described. This compound has been licenced to Artham for development for vascular malformations and to Petra Pharma for oncology. Artham plans a phase II trial in 2021 while Petra Pharma plans a phase Ib/II trial in solid tumours with *PIK3CA* or *KRAS* mutations in combination with a sodium-glucose transport protein 2 (SGLT2) inhibitor (NCT04073680), based on a concept published by the Cantley group[12] (see below).

MEN1611 (CH5132799) (Figure 2g) is a less selective PI3Kα inhibitor with acceptable human pharmacokinetics [133] that is in Phase 1b/2 clinical trials for breast and colorectal cancers[134].

Specific inhibition of one of the *PIK3CA* hot-spot mutants in a manner that spares the unmutated PI3Kα in non-cancerous cells is a tantalising prospect, however, this has not yet been achieved in practice.

Insights and opportunities

PI3Kα inhibitors in breast cancer—Future efforts in this area will focus on better patient selection, expansion into other breast cancer types and novel combinations. Given that *PIK3CA* mutations are common across the different types of breast cancer, including triple-negative breast cancer (TNBC)[135], there is an interest in clinically exploring PI3K inhibition beyond $HR^+/HER2^-$ breast cancer (reviewed in Refs.[136,137]).

It may also be possible to refine *PIK3CA*-based patient stratification strategies, for example by assessing the presence of composite *PIK3CA* mutations (which have been shown to render cells more sensitive to PI3Kα inhibition[58]) or mutant *PIK3CA* gene copy number[68]. Indeed, in a recent clinical study with the AZD5363 AKT inhibitor, homozygosity of the $AKT1^{E17K}$ mutation was associated with an improved therapeutic response[138]. Similar data have been shown for Ras, where cells with multiple copies of mutant Ras are more sensitive to MAP kinase inhibition[139]. Predictive biomarkers beyond *PIK3CA* status could include a transcriptional PI3K pathway activity score[140] or FOXM1 expression[141]. The latter has been reported as a biomarker of both response and resistance to PI3Kα inhibition in ER-positive *PIK3CA*-mutant breast cancer, with FOXM1-driven expression of lactate dehydrogenase allowing a targeted metabolic tissue imaging approach[141].

Following the approval of alpelisib, multiple trials testing additional combinations with hormone therapy and other agents in breast cancer are now in progress or have been opened. *PIK3CA* mutation has been implicated in resistance to fulvestrant-CDK4/6 inhibitor combination therapy[142,143]. This is the basis for the BYLieve trial (NCT03056755) to test alpelisib in combination with hormone therapy in this population of previously-treated breast cancer patients. Conversely, the finding that the CDK4 pathway also mediates resistance to PI3K inhibitors in *PIK3CA*-mutant preclinical models[144], has lead to trials evaluating the combination of inavolisib with endocrine therapy and palbociclib (CDK4/6 inhibitor) in breast cancer (NCT04191499). Another combination of alpelisib is with chemotherapy. Around 10% of TNBC are *PIK3CA*-mutant, further enriched in patients with apocrine or luminal tumors[145]. In patients with metastatic TNBC, the EPIK-B2 trial (NCT04251533) is comparing paclitaxel to paclitaxel plus alpelisib in patients with *PIK3CA* mutation.

PIK3CA mutations also occur in around 30% of breast cancer with amplification of the ERBB2-gene[146], the target of the trastuzumab/herceptin anti-HER2 antibody, and PI3K pathway alterations have been associated with resistance to trastuzumab[147]. Based on these data, a phase III randomized trial has started to compare maintenance anti-HER2 therapy with or without alpelisib in patients with *PIK3CA*-mutant ERBB2-amplified breast cancer (NCT04208178). Based on the observation that involisib preferentially degrades mutant p110α protein in HER2-amplified cells[130], this PI3K is now being tested in $HER2^+$ breast cancer in combination with a range of endocrine therapies or targeted agents such as a CDK4/6 inhibitor, metformin and HER2 antibodies (NCT03006172; NCT04802759).

PI3Kα inhibitors in cancer beyond breast—Additional therapeutic opportunities for PI3Kα inhibitors beyond cancer include PROS, obesity and metabolic syndrome (BOX 2; Table 3). However, given that PI3Kα inhibition is mainly utilised in cancer, opportunities in this setting are described in more detail below (Table 3).

The mechanism underpinning PI3K inhibitor and hormone combination therapy in breast cancer is compelling (Figure 5b). The mechanistic rationale for other combination approaches with PI3K inhibitors such as with chemotherapy, radiation and targeted therapy is not always entirely clear, other than the obviously clinically important observation that resistance against these therapies can be overcome by PI3K inhibitors in preclinical studies[3].

A combination approach with a clear mechanistic rationale is provided by the finding that PI3K inhibition can inhibit homologous recombination through downregulation of BRCA1/2 expression, leading to increased DNA damage and enhanced poly ADP-ribosylation, resulting in sensitization to PARP inhibitors[148,149]. Evidence has been presented that this downregulation of BRCA1/2 gene expression is due to ERK-dependent activation of the ETS transcription factor[148,149]. In addition, the PI3K pathway is key in the production of nucleotides for DNA synthesis, the synthesis of which could be blocked by PI3K inhibitors, which could be problematic for cells under conditions that require DNA repair such as when BRCA1/2 levels are low. Based on this rationale, alpelisib has been combined with the PARP inhibitor olaparib in a phase I trial (NTC01623349). This combination was found

to be feasible and led to 34% objective responses in *BRCA1* wild-type ovarian cancer patients[150].

Around 15% of gastric cancers present a *PIK3CA* mutation, with *PIK3CA* mutations being enriched in EBV-positive subtypes[151]. A phase I/II trial is currently testing the combination of alpelisib with paclitaxel in this molecular subgroup (NCT04526470). In addition, *PIK3CA* mutation/amplification is found in 21% of head and neck cancers, and in 56% of the HPV^+ subset of head and neck cancers[152]. The combination of paclitaxel and alpelisib in patients with head and neck cancer is currently being evaluated (NCT02051751). Combination of radiation with alpelisib[153] or GDC-0032[154] has also shown promising results in preclinical head and neck cancer studies.

An upcoming trial (NCT04073680) will test the combination of PI3Kα and SGLT inhibitors in solid tumours. Indeed, PI3K inhibitors reduce glucose uptake in insulin-responsive tissues such as muscle and adipose, leading to an excess in circulating glucose. This results in a compensatory insulin release from the pancreas which partially negates the anti-tumour effects of PI3K inhibition in cancer cells[12]. SGLT inhibition, which helps to reduce systemic glucose levels by blocking re-uptake of glucose by the kidneys from the urine into the blood, enhances the anti-cancer effect of PI3K inhibitors in preclinical models[12].

Anti-angiogenic and immunogenic effects of PI3Kα inhibition—Given the ubiquitous expression of PI3Kα, its inhibition is expected to affect the tumour stroma, including fibroblasts and endothelial cells[155]. PI3Kα blockade can dampen or normalize tumour angiogenesis in preclinical models[156,157], which might be achieved at PI3Kα inhibitor doses which do not affect the tumour cells themselves[157], similar as observed with low doses of the RAD001/everolimus mTORC1 inhibitor[158]. PI3Kα inhibitors would also dampen the paracrine, potentially tumour-promoting effects of *PIK3CA*-mutant cells discussed above.

Isoform-selective PI3Kα-inhibitors have little or no effect on lymphocytes[131] and other leukocyte types, and are therefore expected to not *directly* affect the immune response. However, evidence is emerging that PI3Kα inhibitors can *indirectly* modulate the immune response, in line with emerging evidence that drugs, initially developed to target the cancer cells themselves, also have immunomodulatory effects that can be exploited in cancer therapy (reviewed in Ref.[159]). PI3Kα inhibition by BYL719, especially in combination with CDK4/6 inhibitors, altered the cytokine and increased expression of MHCI/II proteins on the cancer cell surface, which may increase antigen presentation of tumour antigens[160]. These data are in line with emerging evidence that PI3K inhibition enhances the induction of cell surface MHC I and II molecules by IFNγ[161,162]. The triple combination of BYL719, CDK4/6 inhibition and immune checkpoint inhibitors induced complete and durable tumor regression of a syngeneic TNBC cancer model in mice, correlating with increased activation of both adaptive and innate immunity and a decreased frequency of immune-suppressive MDSCs within the tumour environment[160].

Additional indirect ways of immunomodulation by PI3Kα inhibition might result from a reduction in glucose utilisation by the cancer cells[101,102], leading to enhanced availability

of metabolic fuels in the stroma for immune cells[103], and thus remove tumour-induced metabolic constraints on immune cells (Figure 6a). PI3Kα inhibitors could also modulate the immunomodulatory secretome induced by *PIK3CA* expression[81].

Overall, these data are in line with observations mentioned above, that pulsatile pan-PI3K inhibition with copanlisib or BAY1082439 in a range of preclinical syngeneic cancer models induces favourable anti-tumour immunomodulatory effects[163]. Similar data have been reported with the PI3Kα/β/δ inhibitor BAY1082439 in PTEN-null tumour models[164]. Evidence for induction of favourable immune profile changes by AKT inhibitors in breast cancer has also been reported[165].

PI3Kα inhibitor dosing regimens—Thus far, PI3K-targeted therapies in cancer have been mainly based on the principle of continuous drug dosing at the maximum-tolerated dose defined in phase I trials. Alternative dosing regimens are being explored to increase the tolerability of PI3K inhibitors, while at the same time achieving sufficient PI3K pathway inhibition.

In a preclinical study in mice, encapsulation of alpelisib/BYL719 into P-selectin-targeted nanoparticles led to drug accumulation in the tumour milieu, resulting in tumour growth inhibition and radiosensitization at lower doses of BYL719 compared with oral administration, and without inducing the metabolic side effects normally observed after BYL719 treatment[153].

Intermittent dosing is another approach to improve PI3K drug tolerance. This is illustrated by the PI3Kα inhibitor serabelisib: in phase I studies, only intermittent dosing led to an acceptable safety profile and also enabled higher doses and total weekly exposures as compared to once-daily dosing[132]. An intermittent dosing schedule is also being used for the pan-PI3K inhibitor copanlisib[166,167] which may be feasible due to the long half-life of copanlisib as a result of high volume of distribution and low clearance. In animal studies, copanlisib has shown marked accumulation in tumours over plasma[167]. This accumulation has been ascribed to the sequestration of the basic copanlisib molecule in acidic tumour tissue. If true clinically, this would mean that all four class I PI3K isoforms were inhibited in tumour tissue over the entire dosing interval.

Interestingly, intermittent dosing can, at least in part, convert the cytostatic effect of PI3K inhibitors into a cytotoxic one, with pulsatile dosing of the PI3Kα/δ inhibitors GDC-0941[168] or AZD8835[113] or the pan-PI3K inhibitor copanlisib[112] inducing some level of tumour cell apoptosis in xenograft studies. While the therapeutic impact of such single-agent PI3K inhibitor dosing remained modest[112,113,168], this approach may be better tolerated and allow drug combination therapies.

PI3K-based therapy assumes that all cancer-promoting effects of PI3K are reversible. However, while *PIK3CA* mutation might be critical at certain stages during cancer evolution, for example to tolerate the negative impact of ongoing chromosomal instability[98], it may no longer be required once the cancer cell has adapted to its new genomic configuration. Such a role of genetic *PIK3CA* activation could be exploited by using PI3K

pathway inhibitors to dampen cancer progression and evolution at any stage, and would be expected to be most effective in tumours with clonal PI3K activation such as breast cancer. Importantly, this might be achievable at lower drug doses than the maximum-tolerated doses of PI3K inhibitors currently used in the clinic[99].

Interestingly, pulsatile pan-PI3K inhibition with copanlisib or BAY1082439 in a range of preclinical syngeneic cancer models induces favourable anti-tumour immunomodulatory effects[163,164]. A key question is whether such effects could also be achieved by PI3Kα-selective inhibitors.

PI3Kδ: from biology to approved drugs

PI3Kδ in health and disease

The highly-enriched expression of PI3Kδ in all leukocyte types has endowed this PI3K with roles in immunity and haematological malignancies. These functions are summarized below, and have turned out to be highly intertwined in the clinic.

PI3Kδ in immunity—Preclinical studies using p110δ KO/KI mice and early-generation PI3Kδ inhibitors[169] revealed roles for PI3Kδ in diverse immune functions, suggesting the potential for PI3Kδ inhibitors in autoimmune and inflammatory disorders[5,6,170-173], and allowing the development of cell-based assays for PI3Kδ drug development programmes. These include B-cell activation assays[174-176] and anti-IgE-mediated basophil degranulation tests[177], which have also been used to monitor the impact of PI3Kδ inhibition in whole blood assays from patients (as exemplified in Ref.[32]).

PI3Kδ is functionally-dominant in lymphocytes whereas PI3Kγ plays a more important role in myeloid cells, although this distinction is not absolute[5]. In the context of an *in vivo* immune response, leukocytes are confronted with a range of concurrent stimuli acting through different receptor mechanisms, with PI3Kδ and PI3Kγ often working together to generate a functional output, first documented in neutrophils[178]. Such partnership of PI3Kδ extends to PI3Kβ, as illustrated by the cooperation of these PI3Ks in neutrophil activation by immune complexes[179].

Although PI3Kα plays a minor role in lymphocyte signalling[131,180], it compensates for PI3Kδ inhibition in B-cell development in mice[131,181] and in human B-cell malignancies[182,183]. Interestingly, PI3Kβ is expressed at very low levels in B-cells[181].

PI3Kδ mutation in immune disorders—Patients with homozygous bi-allelic deletion or loss-of-function mutations in *PIK3CD* demonstrate various forms of immunodeficiency, characterised by a profound block in B-cell development and a range of immune dysregulatory diseases including sinopulmonary infections, opportunistic pneumonias, inflammatory bowel disease, autoimmune hepatitis and juvenile idiopathic arthritis[184-188]. Bi-allelic loss of *PIK3R1* (p85α) has also been reported and leads to a block in B-cell development[189].

The first report of a *PIK3CD* mutation in humans with immunodeficiencies was published in 2006, but the functional impact of this E1021K mutation on PI3Kδ was not assessed at the time[190]. Heterozygously-expressed, activating germline mutations in *PIK3CD* are now known to cause the Activated PI3Kδ Syndrome (APDS)[191] primary immunodeficiency, also known as PI3Kδ-activating mutation causing senescent T-cells, lymphadenopathy and immunodeficiency (PASLI)[192 193-195].

Splice site mutations in *PIK3R1* that lead to skipping of exon 11 resulting in a small in-frame deletion (amino acids 434–475) of the p85α inter-SH2 domain, result in a clinical phenocopy of APDS/PASLI, referred to as APDS2. This deletion ablates some of the structural inhibitory activities of p85 on the p110 subunits, leading to their de-inhibition[196,197]. These mutant p85α proteins preferentially activate p110δ and not p110α or p110β[196,197], and therefore mainly act in the immune system. p85α proteins are ubiquitously expressed, and the selective immune impact of APDS2 mutations may relate to the observation that p110δ, compared to p110α and p110β, preferentially associates with p85α over p85β[198].

Immune-related defects in APDS patients frequently include lymphadenopathy and sinopulmonary infections, with an increased predisposition to autoimmune and inflammatory complications, and lymphoma[193,195]. APDS patients present symptoms of both immune deficiency and autoimmunity, indicating the need for a careful balancing of organismal PI3Kδ signalling, with too little or too much PI3Kδ activity having a deleterious immune impact[193,199,200].

Several mouse models with APDS mutations have been generated[199-205], providing further insight into how unbalanced PI3Kδ activity leads to immune dysregulation.

PI3Kδ in B-cell lymphoma—PI3Kδ was positioned as a potential drug target in haematological malignancies, particularly B-cell malignancies, based on high PI3Kδ expression in B-cells and defects in B-cell development and function being the most apparent phenotype in mice with inactive PI3Kδ[174-176].

Mutational activation of PI3Kδ is a rare event in haematological malignancies. However, the E1021K mutation in *PIK3CD*, which is functionally equivalent to the H1047R mutation in *PIK3CA*[191], is present at low frequency in diffuse large B-cell lymphoma[206] and T-cell acute lymphoblastic leukemia[207].

Although *PIK3CD* is not mutated in CLL and FL, these cells show constitutive PI3K pathway activation as a consequence of chronic B-cell antigen receptor (BCR) activation and microenvironmental stimuli[208-210]. This is likely the basis for the superior clinical impact of PI3Kδ inhibition in this setting, compared to other haematological malignancies, as detailed below.

PI3Kδ inhibition in cancer

3-pronged action of PI3Kδ inhibition—In B-cell lymphoma, the therapeutic impact of PI3Kδ inhibition derives from a dual, most likely triple, mode-of-action (Figure 6a,b).

The first is a cancer-cell intrinsic impact, a key factor being that some B-cell malignancies (such as CLL and FL) remain highly dependent on PI3Kδ, similar to non-transformed B-cells. Such reliance of cancer cells on a single signalling pathway, and in this case on a single PI3K isoform is rare, and not observed in other cancer contexts[211]. This creates a unique vulnerability specifically in the B-cell malignancies in which the BCR is required for maintenance and survival. In the B-cell malignancies for which PI3Kδ inhibitors have been approved, there is no correlation between clinical drug efficacy and previously defined high-risk genetic groups. Similar to PI3Kα inhibitors, multiple cellular resistance mechanisms have been described (BOX 1).

Other than depending on PI3Kδ, BCR signalling is also regulated by the BTK, LYN and SYK tyrosine kinases[212]. Although blockade of BCR signalling is considered to be key to the therapeutic impact of inhibitors of these kinases, evidence is emerging that each of these inhibitors shows a distinct, pleiotropic mode-of-action in cancer therapy that does not fully overlap with that of PI3Kδ inhibitors[213 212]. PI3Kδ inhibition also interferes with the response of malignant B-cells to a range of cytokines, chemokines, co-stimulatory molecules and adhesion receptors, which support leukaemic cell maintenance and homing. Several of these stimulatory factors are provided by the surrounding stroma.

A likely second element of the anti-cancer activity of PI3Kδ inhibition is a direct negative impact on leukaemia-supporting stromal cells, and counteraction of microenvironment-derived proliferation and survival signalling pathways. This is best documented in CLL[214] and FL[215] , which both exist in complex niches containing a range of cancer-supporting cell types.

In CLL, these stromal cells include myeloid-derived nurse-like cells and mesenchymal fibroblast-like cells[214,216,217] (Figure 6a). PI3Kδ inhibition in these PI3Kδ-expressing cells[217] dampens their capacity to provide leukaemia-supporting signals. Leukaemia-associated T-cells can also be leukaemia-promoting by providing CD40 ligand (CD40L), IFNγ, and other stimulatory agonists for CLL cells (Figure 6a). Treatment of CLL cells *in vitro* with idelalisib abrogates signalling and survival induced by CD40L or TNFα[208]. Treatment of normal T-cells in *vitro* with idelalisib reduces production of IL-6, IL-10 and TNFα[208]. In patients, decreases in circulating cytokines and chemokines produced by both the CLL and stromal cells are observed following initiation of PI3Kδ inhibitors[209,218] (Figure 6a). Disruption of the stroma-tumour interactions most likely underlies the characteristic lymphocytosis observed using PI3Kδ inhibition in CLL whereby both normal lymphocytes and leukaemic cells leave their lymph node and bone marrow niches to enter the circulation, resulting in an increase in the circulating white blood cell count upon initiation of therapy. The release of leukaemic cells from their protective niches into the blood is expected to increase cell death from loss of survival-promoting stimuli and render these cells more vulnerable to combination therapy, such as with anti-CD20 antibodies or bendamustine chemotherapy.

FL is also characterized by a strong dependence on micro-environmental cues provided by proliferating B-cells and a broad range of supportive cells, including several types of follicular T-cells: T follicular helper (TFH) cells with strong pro-survival activity

through the CD40L/IL4 axis, immunosuppressive T follicular regulatory (TFR) cells, follicular reticular cells, tumor-associated macrophages (TAMs) and follicular dendritic cells (FDCs) displaying antigen presentation (Figure 6b). Notably, both TAMs and FDCs express lectins that activate stereotypic mannosylated residues in the BCR of FL cells, leading to autonomous signalling[219]. PI3Kδ inhibition interferes with this tumour-promoting FL micro-environmental crosstalk, including disruption of FDC-induced angiogenesis and dissemination cues, TFH-induced proliferation and recruitment of Treg cells via downmodulation of CCL22. The overall result of PI3Kδ inhibition is a less supportive and tolerogenic immune microenvironment (Figure 6b)[215].

A third facet of the anti-cancer action of PI3Kδ inhibition is a potential host anti-leukaemia immune response (Figure 6a,b). Indeed, an unexpected observation was that systemic PI3Kδ inhibition in preclinical cancer models in mice, including in leukaemia, leads to enhanced anti-tumour immune responses[220-228]. Pharmacological inactivation of PI3Kδ showed similar effects, also on tumour cell lines resistant to the *in vitro* anti-proliferative effect of PI3Kδ inhibitors and/or which do not express PI3Kδ[224]. The contribution of a host immune response to the anti-tumour effect of PI3Kδ inhibition in leukaemic patients remains to be determined. This potential of PI3Kδ inhibition is being explored in cancer immunotherapy in solid tumours (see below).

Mechanistic studies revealed that PI3Kδ inactivation allowed mice to raise an adaptive anti-tumour immune response, through preferential inhibition of the immunosuppressive regulatory T-cell (Treg) population over the $CD4^+$ T-helper cells and $CD8^+$ T-cells[224] (reviewed in Ref.[229]), leading to a 'rebalancing' of the adaptive immune system towards a $CD8^+$ T-cell response (Figure 6c). This preferential inhibition of Treg cells upon PI3Kδ blockade was subsequently confirmed in other studies in mice[220,222,223,226,227,230,231], in *ex vivo* human T-cell subpopulations from healthy donors[232], in idelalisib-treated CLL patients[232,233] (with patients who experience toxicity displaying a trend towards a lower Treg percentage and a lower Treg:CD4 ratio compared to patients without toxicity[233,234]) and most recently in a phase II trial in head and neck cancer (AMG-319; NCT02540928)[235]. At present, it is not clear why Tregs are more sensitive to PI3Kδ inhibition compared to other T-cell populations. This differential impact on T-cell populations is most likely complex, similar to the impact of PI3Kδ inhibition on $CD4^+$ T-cell differentiation in mouse models which leads to both immunodeficiency and immune activation, in a context-dependent manner[236].

PI3Kδ inhibition also dampens the activity of cancer-promoting myeloid-derived suppressor cells (MDSCs)[224] and cancer-associated macrophages[237], which also reduces the capacity of the latter cells to produce reactive oxygen species that result in the death of Natural Killer cells[238]. It is therefore likely that regulation of both the adaptive and innate immune system underlies the host anti-cancer immune response induced by PI3Kδ inhibition.

Clinical development of PI3Kδ inhibitors

Approved PI3Kδ inhibitors—IC87114, the first PI3Kδ-selective ATP-competitive small molecule inhibitor, was reported in 2003 (Ref.[169]). Studies with IC87114 and the first clinical candidate CAL-101 (Calistoga) provided some evidence for an *in vitro* anti-

proliferative impact of PI3Kδ inhibition in leukaemic cell lines and patient-derived leukaemic cells[30,208,209,239-243]. The initial phase 1 trial of CAL-101 included B-cell malignancies and AML. In line with an important role of PI3Kδ in B-cells, early signs of therapeutic efficacy were mainly observed in B-cell malignancies, particularly in CLL and indolent Non-Hodgkin's lymphoma[218,244]. Response rates were high and responses were durable in heavily-pretreated patients with these diseases.

In 2011, Gilead Sciences acquired Calistoga and continued clinical development of CAL-101 (renamed GS-1101/idelalisib/Zydelig), culminating in its approval in 2014 for the treatment of CLL, relapsed follicular B-cell lymphoma (FL) and relapsed small lymphocytic lymphoma (SLL), following impressive results for idelalisib monotherapy[245] and combination trials with rituximab, an antibody directed against the CD20 B-cell surface marker[246] (Table 1).

In 2017, the intravenously-administered pan-class I PI3K inhibitor copanlisib (Bayer) was approved for adult patients with relapsed FL, followed by approval of the orally-available dual PI3Kγ/δ inhibitor duvelisib (Verastem) in 2018 for adult patients with relapsed or refractory CLL or SLL as well as those with relapsed FL.

In 2020, the FDA granted a fast track designation to umbralisib in combination with the investigational CD20-directed monoclonal antibody ublituximab for CLL, and in 2021, the FDA granted accelerated approval to umbralisib for marginal zone (MZ) lymphoma and FL.

PI3Kδ inhibitors in development—Inhibitors with improved PI3Kδ-selectivity have now been developed (Table 2), with multiple candidates progressing to clinical studies[7]. The principal focus has been for haematological malignancies, though there has also been considerable interest in treating immunological/inflammatory conditions including rheumatoid arthritis, COPD, allergic asthma, psoriasis, Sjögren's syndrome, allergic rhinitis[247] and airway inflammation[248] (Table 2). The most selective PI3Kδ inhibitors to reach clinical trials are parsaclisib (Figure 4f) and nemiralisib (Figure 3a) and most likely also IOA-244 (iOnctura; Supplementary Figure 1a). A key question is whether drug chemistries other than idelalisib will result in similar toxicities.

Unlike all other PI3K inhibitors, which bind to the ATP site, and can be classified as type I kinase inhibitors, the PI3Kδ inhibitor IOA-244 (iOnctura; Supplementary Figure 1a) is non-ATP competitive. Though not particularly potent, IOA-244 must have a different binding mode representing the first so-called type 4 PI3K inhibitor. The expected consequences of this unique binding mode are the potential for very high PI3K isoform and overall kinase selectivity, with fewer off-target side-effects[17,249]. However, its *in vitro* selectivity is only modest against PI3Kβ (20 fold) and not exceptionally high against PI3Kα (130-fold) and it will be of interest to see the PI3K isoform inhibitor profile of this unique allosteric PI3Kδ inhibitor in patients. This compound started phase I studies as monotherapy or in combination with pemetrexed/cisplatin, focused on solid tumours with high expression of PI3Kδ protein, namely metastatic melanoma, mesothelioma or ocular/uveal melanoma (NCT04328844).

The orally available parsaclisib (Incyte) (Figure 4f) is in multiple trials for B-cell malignancies, and in combination with anti-PD1 antibodies in a range of advanced solid tumour indications (NCT02646748/NCT03589651). Although completed, results of a study of parsaclisib in Sjögren's syndrome (NCT03627065) have not been reported. Incyte is also exploring the use of parsaclisib in myelofibrosis (NCT02718300/NCT04551053/NCT04551066).

Nemiralisib (GSK) (Figure 3a) has been developed for inhaled administration and has pharmacokinetic properties unsuitable for oral or IV use. Nemiralisib showed lack of clinical efficacy in COPD, and its further development is currently on hold. Other inhaled agents under development include the dual PI3Kγδ inhibitor AZD8154 (AstraZeneca; Supplementary Figure 1h)[250] and the PI3K inhibitor CHF6523 of undisclosed profile (Chiesi; Supplementary Figure 1i)[251]; which are being investigated in asthma (NCT04187508) and COPD (NCT04032535), respectively.

Umbralisib (Figure 4e) (TGR-1202; TG Therapeutics)[8] has is progressing in CLL, FL and MZ lymphoma[9]. Notably, MZ B-cell development and residence in the spleen was one of the clearest phenotypes upon genetic or pharmacological inactivation of PI3Kδ in mice[175,176,252]. Umbralisib is highly selective for PI3Kδ and also inhibits casein kinase 1ε[253], both of which are likely to contribute to a different safety profile to that of other PI3Kδ inhibitors, as explained below.

Zandelisib (ME-401; MEI Pharma; **Supplementary Figure 5j**) exhibits high selectivity for PI3Kδ[254], and is in a phase II registration trial, also for FL.

Another advanced agent is leniolisib (CDZ173; Novartis; now licenced to Pharming) (Figure 3b), which is in phase III for APDS[255] (NCT02435173/NCT02859727). A dose-finding trial with leniolisib found evidence for immune normalisation and overall patient benefit with no discernible adverse effects[255]. The latter might be related to the specific condition of pre-existing overactive PI3Kδ that is being normalised by this targeted drug treatment. Recruitment has started with a larger cohort of up to 30 APDS patients, not insignificant for a rare disease. However, leniolisib did not show clear efficacy in Sjögren's syndrome (NCT02775916)[256]. Seletalisib (UCB-5857) (Figure 4d), another PI3Kδ-selective inhibitor which exhibits good selectivity against PI3Kα and PI3Kβ[32] has also been investigated in Sjögren's syndrome (NCT02610543, Phase II), but development in this indication appears to have ceased[257]. In addition, seletalisib entered a proof-of concept study in APDS1 and APDS2 patients, in which it demonstrated modest activity and significant side effects (liver injury, colitis, infections)[258]. Such adverse effects were not observed in the APDS patients in the trials with leniolisib mentioned above[255], for reasons that are currently unclear.

Other notable agents in development include the PI3Kδ inhibitor YY-20394/linperlisib (Shanghai Yingli Pharmaceutical; structure not disclosed), which is being investigated in both haematological and solid tumours and AMG319 (Figure 4g; Amgen) which has been tested in a window-trial in Head and Neck cancer (NCT02540928)[235].

Several other companies are also exploring the utility of PI3Kδ inhibitors or dual PI3Kγ/δ or PI3Kβ/δ inhibitors in haematological malignancies, as monotherapy or in combination with other targeted agents or chemotherapy (reviewed in Ref.[259]; Table 2).

Another approach to reduce PI3K activity, is by activation of SHIP1. This phosphatase is mainly found in leukocytes and hydrolyses PIP_3 to $PI(3,4)P_2$ (Figure 1). Activation of SHIP1 functionally acts to reduce PI3K signalling which, in leukocytes, is principally mediated by PI3Kγ and PI3Kδ. Activation of SHIP can thus reduce the activity of these two PI3K isoforms. AQX-1125 (rosiptor; Supplementary Figure 1k)[260,261] is an allosteric activator of SHIP1 that was investigated in phase II clinical trials for asthma[262] and other inflammatory conditions, however all development was stopped after a phase III trial for interstitial cystitis and bladder pain failed[263].

Challenges and opportunities

Below, we discuss the future of PI3Kδ inhibitors, highlighting approaches to address the key challenges of drug toxicity and tolerability, and present clinically-advanced opportunities for PI3Kδ inhibitors. Preclinical data suggests additional potential therapeutic opportunities that have yet to be tested in the clinic (BOX 3).

Managing PI3Kδ inhibitor toxicities—Immunomodulation by early generation PI3Kδ inhibitors has resulted in adverse effects which hampered clinical progress in this area. These toxicities may relate to the drug characteristics and doses used (compounds not being fully selective for PI3Kδ over other PI3K isoforms, degradation of drugs resulting in metabolites with liver toxicity, use of maximum-tolerated doses which may not be required for interference with tumour:stroma interactions or immunodulation) as well as the patient population tested, with a tendency for toxicities to be lower in heavily-pretreated and/or elderly cancer patients, i.e. people with reduced immune competency.

The incomplete PI3K isoform selectivity of first generation inhibitors for PI3Kδ over PI3Kγ and/or p110β may very well have contributed to the observed toxities of these compounds. In this context, it is of interest to mention that genetic inactivation of both PI3Kγ and PI3Kδ in mice leads to severe autoimmunity and inflammation and was very poorly tolerated[264]. Inactivation of PI3Kδ in mice does not lead to obvious immune-related adverse effects, including auto-immunity, other than the predisposition to colitis[176], which is exaggerated by non-SPF mouse housing conditions[265,266].

Early trials with idelalisib in haematological malignancies enrolled mostly heavily-pretreated patients having had more than one prior treatment with chemotherapy. In these populations, the adverse effects were mostly manageable, and included infections at typical rates seen in those patients, as well as relatively rare and likely immune-related colitis, hepatotoxicity and pneumonitis [233,267,268] which were responsive to steroids. In follow-up trials in treatment-naïve and often younger patients, idelalisib was associated with more severe autoimmune adverse effects that were treatment limiting[233], [269]. Adverse effects observed upon PI3Kδ inhibition, mainly observed using idelalisib, included bacterial infections (possibly in part secondary to drug-induced neutropenia), opportunistic infections (fungal infections and reactivation of cytomegalovirus (CMV)

and inflammatory/autoimmune toxicities including colitis, hepatotoxicity/transaminitis and pneumonitis[233,267,268], which may result from an overactive immune response in tissue locations exposed to external immunogens (skin, lung, bowel and liver). Given the complex immune impact of PI3Kδ inhibition, with elements of both immune suppression and activation[236], explaining the adverse effects observed is challenging. At the time of these trials, the immuno-modulatory activities of PI3Kδ inhibition were under-appreciated, as were the potential toxicities of combination therapies using novel agents[270]. Retrospectively, it would have been advisable to mandate antimicrobial prophylaxis in idelalisib trials[271]. This is standard procedure when testing immunomodulatory agents in potentially immune-compromised patients but was not mandatory in these trials.

There has also been speculation that the primary liver metabolite of idelalisib, GS-563117[272], an inhibitor of the CYP3A cytochrome P450 isoform, could be a possible cause of toxicity in this organ. This was also suggested for another idelalisib-glutathione (GSH) adduct[273]. Evidence has been presented for a differentiated safety profile of parsaclisib relative to idelalisib and duvelisib, with a near absence of grade ≥2 transaminitis/hepatotoxicity[230,274]. On the other hand, an individual who had congenital loss of both *PIK3CD* alleles was reported to have autoimmune hepatitis[187], arguing that clinical toxicity of PI3Kδ inhibitors may be at least partially due to an on-target effect of PI3Kδ inhibition, potentially exacerbated by activated $CD8^+$ T-cells (Figure 6). Additionally, in the clinical study of idelalisib with ofatumumab in which early fulminant hepatotoxicity was seen, liver biopsies were performed in two patients, both of whom showed a liver infiltrate with activated $CD8^+$ T-cells[268]. Increased transaminitis levels were also correlated with reduced Treg[233]. Furthermore, recurrence of transaminitis with resumption of idelalisib was blocked by concomitant corticosteroids, consistent with an immune-mediated mechanism.

An interesting observation is that the PI3Kδ inhibitor umbralisib (TG Therapeutics) appears to lead to fewer adverse events such as colitis compared to idelalisib and duvelisib[8,275]. This may relate to the fact that (1) umbralisib is very selective for PI3Kδ over PI3Kα and PI3Kβ and has an approximately 225-times greater selectivity over PI3Kγ (Table 1); (2) umbralisib, unlike most other kinase inhibitors, is not metabolized through the classical cytochrome P450 3A4 (CYP3A4) metabolic pathway[276], and that (3) umbralisib also inhibits casein kinase 1ε (CKε)[253]. Inhibition of CKε on its own *improves* CLL Treg number and function[277]. CKε inhibition by umbralisib may therefore protect Tregs from the inhibitory effects of PI3Kδ blockade[277]. A better preservation of the number and function of Treg cells in CLL patients may translate to reduced immune-mediated side effects of umbralisib compared to other PI3Kδ inhibitors.

PI3Kδ inhibitor treatment is now considered a safer treatment option than at the time of the early clinical trials[259]. Antimicrobial prophylaxis as well as CMV monitoring has now been included in the guidelines for clinical use of PI3Kδ inhibitors[271]. Frequent monitoring for early treatment-emergent neutropenia, with the option to employ growth factors to correct it, is also indicated. Colitis, diarrhoea and transaminase elevations are now often manageable with dose interruption/reduction or drug discontinuation, particularly if identified early, and by the use of corticosteroids, including either systemic or non-absorbable budesonide for colitis[278].

Recent analysis indicated idelalisib monotherapy to be suitable for heavily-pretreated relapsed/refractory FL patients, given the unmet need in these patients[279]. This conclusion is based on a *post hoc* subgroup analysis of such patients enrolled in an idelalisib monotherapy trial[245] in which the benefits in increased PFS were considered to outweigh the safety concerns in this setting[279].

Another reported potential side-effect of PI3Kδ inhibition is induction of genomic instability in B-cells, through activation of AID (activation-induced cytidine deaminase) which promotes DNA recombination[280]. These observations raised concerns about a potential mutagenic risk in patients upon long-term PI3Kδ inhibitor therapy. The extent of this biological effect in the clinic is unclear – particularly as many patients discontinue due to toxicity after short treatment times - and it remains to be determined if these biological activities are found in clinical CLL samples. Indeed, similar effects on AID of the BTK inhibitor ibrutinib, first documented in normal and neoplastic B-cell lines, are not mirrored in primary CLL samples[281]. It is also possible that the anti-tumour effects of PI3Kδ inhibition will outweigh this potential negative side effect.

Improving PI3Kδ inhibitor dosing regimens—Finding tolerable drug dosing regimens is a key challenge to the further development of PI3Kδ inhibitors, together with the generation of inhibitors with improved PI3Kδ isoform-selectivity.

Auto-immune and inflammatory diseases likely require lower PI3Kδ inhibitor doses than in cancer. Lower drug doses may still enable patients to mount immune responses to exogenous immunogens, as illustrated in mice where low doses of PI3Kδ inhibitor were shown to be effective in genetic models of auto-immunity, including the NOD model of type 1 diabetes, without fully inhibiting T-cell responses[282]. Such dosing could be intermittent, whereas for APDS which is associated with permanent genetic PI3Kδ activation, continuous dosing appears the most sensible approach.

Administration of PI3Kδ inhibitors in B-cell malignancies was initially based on the principle of continuous dosing at the maximum-tolerated drug dose, defined in standard dose finding phase I trials and based on the rationale to block as much cancer-cell intrinsic PI3Kδ as possible. However, these doses are not well-tolerated long-term, and guidelines for the management of adverse events associated with idelalisib treatment in B-cell malignancies are now available[267,271,278], with data showing no adverse clinical impact of dose interruptions. Indeed, PFS was significantly improved in FL and CLL patients who had 1 or more treatment interruptions compared to those with none, as long as time off therapy was <8%, with overall survival also improved in CLL patients[283]. This finding may in part be related to duration on therapy, although it also raises the possibility that these patients exhibiting toxicity are also developing an adaptive anti-tumour response (Figure 6), as described above.

Companies are now adapting PI3Kδ inhibitor dosing and scheduling regimens, which might also facilitate combination therapies[284]. Non-continuous/intermittent dosing of parsaclisib and zandelisib/ME-401 (MEI Pharma) in B cell malignancies, has been reported to lower the toxicity profile of these agents[274,285].

Inflammatory/autoimmune diseases—The complex immune impact of PI3Kδ inhibition, inducing elements of both immune activation and suppression, creates a challenge for chronic systemic PI3Kδ inhibition. It is possible that low doses of inhibitor are required, in order to retain overall immune responsiveness, as illustrated in a preclinical study[282]. Topical routes of drug administration have also been explored, for example by inhalation of the PI3Kδ inhibitor nemiralisib (GSK) for inflammatory airway diseases[286]. However, the development of this compound in this disease indication has now been terminated due to lack of clinical efficacy. The dual PI3Kγ/δ inhibitor duvelisib also failed to meet its primary endpoint in allergic asthma (NCT01653756) and in rheumatoid arthritis (NCT01851707)[287]. Further clinical development in immune dysregulation focuses on alllergic asthma (AstraZeneca, with the dual PI3Kγ/δ inhibitor AZD8154; NCT04187508) and COPD (Chiesi; with CHF-6523, a PI3K inhibitor with undisclosed PI3K isoform selectivity; NCT04032535).

B-cell malignancies—The development of PI3Kδ inhibitors for B-cell malignancies fits with the quest for non-chemotherapy-based therapies for these diseases. The efficacy of PI3Kδ inhibitors appears to be tightly linked to dependence of CLL and FL on chronic BCR signalling which is critically dependent on PI3Kδ, and is likely to be more limited in more aggressive B-cell malignancies that have activated additional cell survival pathways.

Given that most patients have partial remissions with monotherapy kinase inhibitors, interest in defining combination therapies that enable deeper remissions and discontinuation of therapy is high. While some combinations are likely safe, such as with antibodies to CD20 in relapsed CLL[288], others, particularly with chemotherapy, are not without their risks[270], especially in treatment-naïve patients[268]. PI3Kδ inhibition restores the dependence of FL cells on the anti-apoptotic protein BCL2[215], providing a rationale for combined PI3Kδ and BCL2 inhibition.

It is now well-established that many CLL patients, despite showing responsive disease upon treatment with kinase inhibitors, often discontinue treatment because of adverse effects. Having access to drugs with differential toxicity profiles allows clinicians to switch these so-called kinase inhibitor-intolerant CLL patients to other drugs. An example is the use of umbralisib in CLL patients who have become intolerant to BTK inhibitors or other PI3Kδ inhibitors[276]. Moreover, as mentioned above, survival benefit is still observed upon treament interruption[283], indicating that effective PI3Kδ inhibitor therapy might not require continued drug administation in order to be clinically effective.

Cancer immunotherapy of solid tumours—The main rationale for use of PI3Kδ inhibitors in solid tumours is the potentiation of an adaptive anticancer immune response (Figure 6c). The high expression of non-mutated PI3Kδ in some solid tumours such as melanoma or breast[289] has recently also received renewed attention, with evidence emerging from xenograft studies in mice that cancer cell-intrinsic PI3Kδ may provide sensitivity to PI3Kδ inhibition[237,290] (reviewed in Ref.[291]).

A report currently under peer-review of a window trial in head and neck cancer using AMG-319, has provided formal evidence for the cancer immunotherapy potential of PI3Kδ

inhibition in human solid tumours[235]. This trial evaluated biomarkers suggestive of anti-tumour immune responses and, consistent with the results obtained in mice[221,224,229], AMG-319 decreased tumour-infiltrating immunosuppressive Treg cells and caused heightened cytotoxic potential of tumour-infiltrating $CD8^+$ and $CD4^+$ T cells[235]. This also led to immune-mediated adverse effects, including skin rash suggesting that alternative dosing regimens will be required to effectively and safely exploit the immunomodulatory impact of PI3Kδ inhibition in human solid cancers. These adverse effects were distinct from and more severe than those experienced by AMG-319-treated lymphoma patients who had undergone prior therapies, suggesting that patients who have not previously been treated with immunosuppressive chemotherapy are more sensitive to PI3Kδ inhibitors[235].

The immunomodulatory dose of PI3Kδ inhibitors is likely to differ from the maximum-tolerated dose often favoured in oncology, and intermittent dosing may also be most effective. Of interest, intermittent administration of the dual PI3Kα/δ inhibitor AZD8835 induces potent immune-mediated anti-tumour responses in syngeneic solid tumour models in mice[292]. Pulsatile dosing of the pan-class I PI3K inhibitors copanlisib or BAY1082439 also generates an effective anti-tumour immune response in a range of animal models[163,164].

At present, there is no evidence to suggest that the break in immune tolerance induced by PI3Kδ inhibition results in *sustained* auto-immunity, as auto-reactive immune symptoms disappear upon termination of PI3Kδ inhibitor treatment, although this can take several months[233,246]. This contrasts with treatment with checkpoint inhibitors which often results in long-term auto-immunity[293-296]. Moreover, in contrast to checkpoint antibodies, which remain in the circulation for weeks, interruption of PI3Kδ inhibitor dosing allows rapid reversal of systemic inhibition, of critical importance upon occurrence of adverse side effects.

A handful of PI3Kδ inhibitors have been/are being studied in solid tumours but it is unlikely that PI3Kδ inhibition will be as effective as a monotherapy. One combination might be with checkpoint inhibitors, which have shown efficacy in preclinical mouse models combining pharmacological PI3Kδ inhibition with anti-CTLA4 or anti-PD1[225,297], with or without radiation[298]. The combination with anti-PD1 is being explored in solid tumours with parsaclisib (NCT02646748/NCT03589651). Given that PI3Kδ signalling might be required for signalling reactivation in exhausted T-cells by checkpoint therapy[299,300], such treatment might be most effective when used *sequentially* rather than *concomitantly*. This is also suggested by the observation that anti-PD1 antibodies do not show effective anti-cancer activity in PI3Kδ-deficient mice[227]. Following the observation of strong upregulation of the immune checkpoint receptor LAG-3 on Treg cells in tumours that escaped the inhibitory effects of PI3Kδ blockade, PI3Kδ inhibitor treatment followed by administration of anti-LAG3 antibodies was found to induce a superior anti-cancer effect in syngeneic mouse cancer models in which PI3Kδ inhibition induced a partial initial anti-tumour response[226].

In mouse models, tumour-infiltrating CSF1-receptor (CSF1R)-positive macrophages neutralize the anti-tumour impact of PI3Kδ inhibition, with combined inhibition of CSF1R and PI3Kδ being effective in inducing an anti-tumour response[228]. Several inhibitors of

CSF1 signalling are being tested in the clinic, and are candidates for combination with PI3Kδ inhibitors.

Other immunotherapy-based avenues that have been explored in mouse models of cancer include combination of PI3Kδ inhibition with tumour-specific vaccines[222,223] or oncolytic viruses[301].

Lastly, PI3Kδ inhibitors could also be used during the expansion of T-cells for adoptive cancer immunotherapy. Indeed, blockade of the PI3K/AKT/mTOR pathway, including of PI3Kδ, during the *in vitro* expansion of T-cells for use in adoptive transfer dampens terminal differentiation of these cells[222,302-310], allowing prolonged expansion in the patient. The underlying mechanism is not entirely clear, but evidence suggests that these inhibitors do not interfere with the *in vitro* proliferation of these T-cells, but instead maintains them in a less differentiated state that is less prone to exhaustion, the progressive loss of effector function.

Concluding remarks

Following the approval of PI3K inhibitors for haematological malignancies, the approval of a PI3Kα inhibitor for solid tumours has heralded a new era in PI3K drug development. Encouraging clinical data are also emerging from ongoing trials with AKT/PKB inhibitors in breast and prostate cancer, in combination with hormone therapy or the anti-microtubule agent paclitaxel[122,138,311,312]. Indications for PI3K inhibitors beyond cancer include overgrowth conditions, obesity and metabolic syndrome, as well as diabetic retinopathy (BOX2; Table 3).

It has become more widely appreciated that PI3K inhibitors are mainly anti-proliferative rather than cytotoxic for cancer cells, at least *in vitro* and in xenograft studies. It is possible, however, that these experimental conditions do not adequately reflect the *in vivo* situation where it cannot be excluded that PI3K inhibitors might lead to the demise of cancer cells due to a combined effect on the tumour cells and the tumour stroma, angiogenesis and the immune system[157].

Emerging evidence suggests that PI3K inhibitors do not need to be administered continuously, and that intermittent dosing might not only be better tolerated but even more effective as an anti-cancer approach. Tolerability of PI3K inhibitors remains an issue, with adverse events including hyperglycemia/diarrhoea for PI3Kα inhibitors[10] and a range of immune-related toxicities and infections for PI3Kδ inhibitors[233,268]. It is clear that improved PI3K isoform selectivity will be key to further development of this class of inhibitors, not least to fully understand their mechanims of action in patients.

For PI3Kα inhibitors, a key advance will be the identification of more tolerable drug dosing regimes and rational-based combination therapies beyond sensitization to hormone therapy in breast cancer, such as combination with PARP inhibitors. Multiple trials with PI3Kα inhibitors are now ongoing or planned.

PI3Kδ biology has turned out to be more complex than anticipated, with organismal PI3Kδ inhibition inducing elements of both immune activation and suppression, creating

a challenge for chronic PI3Kδ inhibition in inflammatory and auto-immune diseases. At present, it is not entirely clear if some of the adverse effects result from co-inhibition by some of these compounds of other PI3K isoforms, especially PI3Kγ. The current utility of PI3Kδ inhibitors may therefore mainly lie in cancer, both in B-cell malignancies and solid tumours. PI3Kδ inhibitors in B-cell malignancies, where there is a strong rationale for both cancer-cell-intrinsic and stromal cancer-supporting roles of PI3Kδ (Figure 6a,b), are currently positioned as a therapeutic option after the failure of other novel agents and/or chemotherapy, because of their toxicity, but can be used safely with careful monitoring and use of prophylaxis[259,313]. However, the development of better tolerated dosing regimens and more effective combination therapies are likely required to bring this therapeutic option back to the forefront of clinical approaches[259]. An exciting observation in CLL patients is that treatment interruptions upon adverse events did not negatively affect clinical impact, and in fact improved overall survival[283]. It is tempting to speculate that at least some of the adverse events are a hallmark of induction of a host immune response, which could be be harnessed as an anti-tumour immune effect including in solid tumours (Figure 6c)[235].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Target therapies
Therapies aimed to selectively modulate the molecules that are deregulated in disease (as opposed to non-targeted therapies, such as for example chemotherapy).

Intrinsic drug resistance
drug resistance that exists in the cells prior to drug therapy.

Acquired drug resistance
drug resistance that develops in cells in response to a drug therapy to which the cells were originally sensitive.

Anabolic metabolism
The cellular process of generation and storage of energy and cell building blocks.

Catabolic processes

The mechanisms of degradation of cellular components, in order to generate energy and recycle building blocks for macromolecules.

Autophagy
A cellular ‘self- eating’ process that involves the sequestering of cytoplasmic material in double-membrane structures (called autophagosomes), followed by membrane traffic to the lysosome for the degradation and recycling of cellular components.

Hotspot mutations
Commonly recurring mutations in a gene in disease, often altering key characteristics or function of the mutated protein.

Apoptosis
A form of programmed cell death, occurring during development and tissue remodellng, in which the cell internally degrades without rupturing the cell membrane, allowing the dead cells to be taken up and degraded by the surrounding cells.

Cell senescence
A highly stable cell cycle arrest which limits the replication of damaged or aged cells.

Epithelial-to-mesenchymal transition (EMT)
A process whereby epithelial cells change their characteristics (such as cell-cell interactions and shape) to become more like mesenchymal cells. EMT is key to embryonic development and is re-activated in carcinoma (cancers derived from epithelial cells), allowing them to become more malignant and to metastasize.

Reciprocal dependency
A condition in which one system influences and depends on another, an vice versa.

Chromosomal instability
A process of ongoing acquisition of genomic alterations, most often in cancer, including the gain or loss of whole chromosomes as well as structural aberrations which range from point mutations to small-scale genomic alterations and gross chromosomal rearrangements.

Secretome
The complement of proteins secreted by cells in the extracellular environment.

Cytostatic
Stopping cells from proliferating, without killing them.

Poly ADP-ribosylation
The process of covalently attaching polymers of ADP-ribose to proteins by poly(ADP-ribose) polymerases (PARPs).

Antigen presentation
The process of displaying peptides by binding these to the major histocompatibilty complex on the surface of antigen-presenting cells, so that these antigens can be effectively recognised and bound by the antigen receptor on the surface of T lymphocytes.

Immune checkpoint
A surface protein of immune and sometimes cancer cells, that helps to stimulate or inhibit the responsiveness of T lymphocytes to antigens.

Micro-environmental crosstalk
Functional interactions between the tumour cells and the cells and tissues that surround around them.

Transaminitis
The presence of high levels of liver transaminase enzymes in the blood, most often a sign of liver dysfunction such as injury or inflammation.

Genomic instability
Increased tendency for the acquisition of alterations in the genome.

Oncolytic viruses
A virus that infects and kill cancer cells, often engineered using molecular biology techniques to optimize these characteristics.

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BOX1 -

Cellular mechanisms of resistance to PI3K inhibitors

PI3Kα inhibitors:

Resistance to PI3K inhibitors is often mediated by feedback loops, a non-genetic acute rebalancing of existing signalling pathways in the cell to neutralise the inhibitory effects, for example through compensatory upregulation of expression of tyrosine kinase receptors[13,14]. *In vitro* treatment of cell lines with alpelisib often leads to compensatory PI3Kβ activation[314,315]. Similarly, treatment with alpelisib of a patient with a *PIK3CA* mutant breast cancer led to recurrent *PTEN* loss in different metastases[316]. In order to overcome such compensatory mechanisms to isoform-selective PI3K inhibitors, so-called 'balanced' pan-PI3K/mTOR inhibitors that block all class I PI3K isoforms and mTOR pathway equally well, continue to be developed[317]. However, given the poor tolerance of such compounds when given systemically, these will most likely have to be administered topically, such as for skin diseases.

PI3Kα inhibitors often have a limited antiproliferative effect in cell lines with inactive PTEN[44,318]. Some of these cell lines have been shown to be instead sensitive to the antiproliferative effect of PI3Kβ inhibitors[318-320], although this correlation is not universal[321] (reviewed in Refs.[322,323]).

A range of resistance mechanisms linked to alterations in PI3K activities or PTEN themselves have also been reported, including amplification of Myc or eIF4E[324], activation of the SGK Ser/Thr kinases (which are highly related to AKT)[325,326], activation of cyclin-dependent kinases CDK4/6[144], persistent expression of FOXM1[141] and loss of negative regulators of mTORC1[327]. One study identified 63 putative alpelisib resistance genes, including activation of the PIM Ser/Thr kinases[328]. A genome-wide shRNA-based screen identified several genes whose suppression could convert the cytostatic effect of PI3K inhibition into a cytotoxic one[109]. Amongst these were the PIM2 and ZAK kinases, small molecule inhibitors of which were found to synergize with PI3K inhibition[109]. Remarkably, no drug-induced resistance mutations in the *PIK3CA* gene itself have been reported.

PI3Kδ inhibitors:

In CLL, primary resistance , i.e. failure to respond at all, may be associated with mutations in the RAS/RAF/MAP2K1 pathway that result in constitutive ERK activation[329]. Unlike for BTK inhibitors, cell lines or CLL tumours with acquired idelalisib resistance do not display unifying recurrent mutations that could be implicated in drug resistance[330,331]. Similar observations were made in a mouse model of PI3Kδ inhibitor-resistant CLL, which showed a very modest increase in acquired mutations (relative to drug-sensitive tumours), with little or no overlap between independently-derived tumours, and no mutations in *PIK3CD* itself[332]. This study suggested that IGF1R overexpression may be associated with PI3Kδ inhibitor-resistant CLL, and demonstrated constitutive ERK activation associated with that overexpression. Other than the likelihood that cancer-cell-intrinsic resistance to PI3Kδ inhibition can be achieved through multiple

mechanisms possibly converging on alternate signalling pathway activation (e.g. ERK), these observations also indicate that the cancer-cell-intrinsic role of PI3Kδ may not be as critical in the observed anti-leukaemic effects of PI3Kδ inhibitors as is the case for BTK inhibitors.

Based on an *ex vivo* co-culture system of FL patient leukaemic cells mixed with FDCs from normal tonsils treated with idelalisib, Serrat *et al.*[215] reported a gene signature that discriminates idelalisib-sensitive from idelalsisib-non-responsive cultures. It will be of interest to test the predictive value of this idelasib-score in clinical trials. This study also reported that idelalisib treatment renders the FL cells sensitive to BCL2 inhibitors, providing a mechanistic rationale for investigating the combination of PI3Kδ and BCL2 inhibition in FL[215].

BOX 2 -

PI3Kα inhibitors in non-oncology indications

PROS

Activating mutations in *PIK3CA*, similar to those in cancer, have been found in benign skin lesions (epidermal nevi and seborrheic keratoses)[333] and in disorders belonging to the *PIK3CA*-related overgrowth spectrum (PROS; reviewed in Ref.[334]). The lack of cancer predisposition in these conditions illustrates the context-dependent impact of genetic PI3Kα activation[88].

In most cases of PROS, *PIK3CA* mutations are acquired postzygotically and thus exhibit tissue mosaicism (i.e. the mutations are not present in all cells). The resulting overgrowth is asymmetric and highly variable, reflecting differences in the timing and location of mutation acquisition during development. Commonly affected tissues include adipose tissue and blood vessels, but also muscle, brain, bone and peripheral nerves[334]. The overgrown tissues often represent a mix of cells with wild-type and mutant *PIK3CA* expression, suggesting potential paracrine effects of *PIK3CA*-mutant cells towards their wild-type counterparts[334] (Figure 5a). Evidence for the capacity of PI3K pathway mutant cells to induce lesion formation in a non-cell-autonomous manner has been reported in an *AKT1*-mutant-driven mouse model of the human Proteus overgrowth syndrome[335]. As mentioned above, *PIK3CA* mutation in cancer cells can result in the secretion of protein and lipid mediators that modulate the biology of surrounding neurons[57], endothelial cells[104] and wild-type cancer cells[100]. Paracrine effects upon loss of PTEN expression have also been reported[336].

Treatment of PROS patients with low doses of alpelisib as part of a compassionate use program has shown a promising clinical impact (Novartis; NCT04085653) with patients experiencing negligible side effects[337]. This contrasts with the observations of a clinical trial of low-dose rapamycin (sirolimus) in PROS which reported only modest clinical benefit, and was associated with a considerable number of adverse effects that led to frequent treatment discontinuation[338].

It remains to be seen if alpelisib treatment will be tolerated in a wider population of PROS patients and alleviate the different tissue overgrowths in PROS to the same extent. A clinical trial with well-defined endpoints has now opened to address these questions (NCT04589650/NCT04085653).

Obesity and metabolic syndrome

While heterozygous genetic PI3Kα inactivation in mice leads to adverse metabolic effects at young age[50], such chronic partial PI3Kα inactivation protects older mice from age-related reduction in insulin sensitivity, glucose tolerance and fat accumulation[339]. Chronic partial pharmacological PI3Kα inactivation also did not lead to major toxicities or side effects in mice[340]. PI3K inhibitors also reduce obesity in mice and monkeys[341,342], attributed to increased energy expenditure as a consequence of activation of thermogenesis in brown adipocytes[343] and increased oxidative phosphorylation together with reduced anaerobic glycolysis[344]. Upregulation of mitochondrial activity

in mouse adipocytes (as well as in *Drosophila* fat bodies)[345] and potentiation of β-adrenergic/cAMP signalling in these cells that leads to increased catecholamine-induced energy expenditure[346], have also been implicated in the beneficial metabolic effects of partial PI3Kα inactivation.

These data indicate that moderate pharmacological inhibition of PI3Kα could be a therapeutic strategy for obesity and metabolic syndrome in humans. While not clear whether this will be tested in a formal clinical trial, it is possible that supportive data will be borne out by clinical trials in other disease settings, especially if these treatments (such as in PROS) would involve long-term administration of low doses of PI3Kα inhibitors (as compared to the maximum-tolerated doses used in a cancer setting).

BOX 3 –

Emerging therapeutic indications for PI3Kδ inhibition

There is preclinical evidence to suggest that PI3Kδ-targeted therapy during the early, acute phase of some infectious diseases (such as *Leishmania*) could be therapeutically useful, exploiting the immunostimulatory impact induced by acute PI3Kδ inhibition through enhanced innate myeloid cell responses and dampened regulatory T and B lymphocyte responses[347]. This fits with the emerging concept of using kinase inhibitors as a 'third arm' in infectious disease, i.e. when antibiotics or vaccines are unavailable or not an option – which is often the case in an acute setting of infection.

Early studies showed that although highly enriched in all types of white blood cells, PI3Kδ can also be present in non-leukocytes[289], mostly at lower levels than in white blood cells and possibly expressed from an alternative promotor which can be activated by inflammatory stimuli such as TNF[348,349]. These include neurons, endothelial cells and fibroblasts, with potential new therapeutic opportunities for PI3Kδ inhibitors. Diverse biological functions of PI3Kδ in these cells have been reported, such as intracellular vesicle trafficking and cytokine production, with possible functional roles in neuronal regeneration[350-352] and schizophrenia[349,353], angiogenesis and immunomodulation in endothelial cells (including in pathological retinal angiogenesis[354,355]) and inflammatory/immunomodulatory functions in fibroblast-like cells such as synoviocytes in arthritic joints[348,356-359]. PI3Kδ inhibition could also have an antitumour effect by suppressing tumour-promoting PI3Kδ-expressing fibroblast-like cells, namely mesenchymal fibroblast-like cells in CLL[216,217] and cancer-associated fibroblasts in breast cancer[359] (Figure 6).

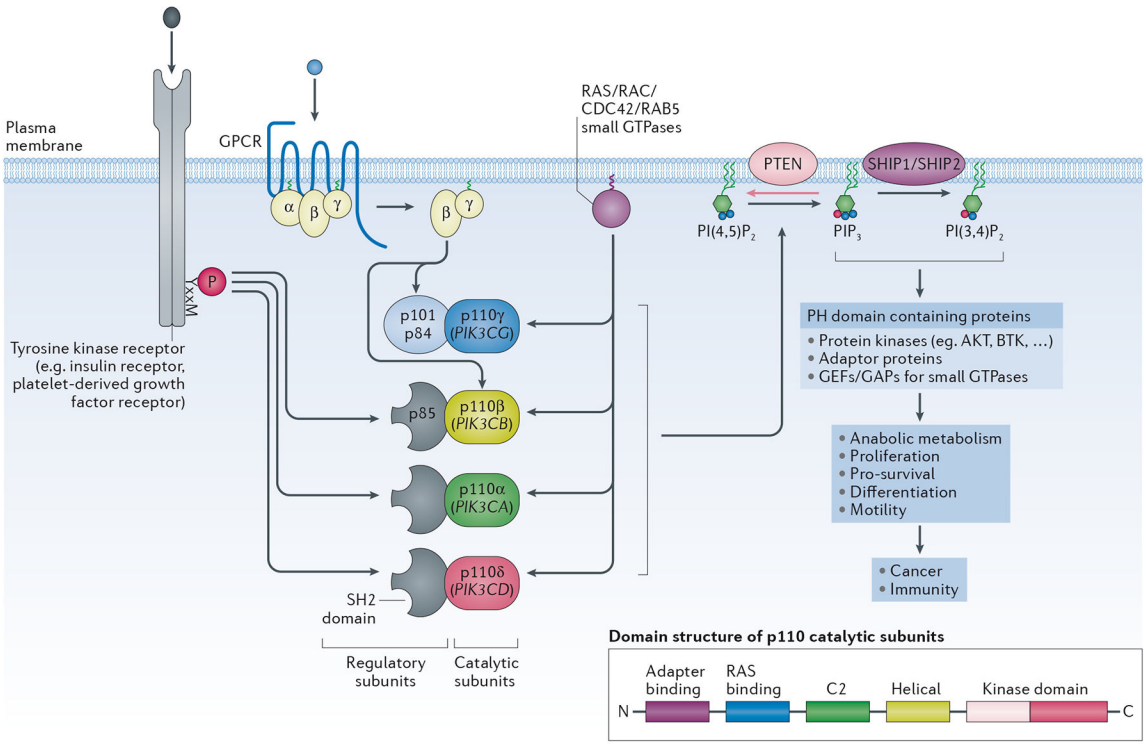

Figure 1 –. General overview of signalling by class I PI3K isoforms.

The class IA PI3K catalytic subunits (p110α, β and δ) bind the p85 regulatory subunits which keep the p85/p110 complex in an inactive, cytosolic form. The p85 subunits have two SH2 domains that allow the p85/p110 heterodimers to bind to phosphorylated tyrosine residues in membrane-associated proteins, such as receptors and adaptor proteins, thereby recruiting the PI3K heterodimer to its lipid substrates while simultaneously disinhibiting its enzymatic activity. Mammals have three genes for p85 regulatory subunits, namely *PIK3R1* (encoding p85α, p55α and p50α), *PIK3R2* (encoding p85β) and *PIK3R3* (encoding p55γ). p110γ, the sole member of the class IB PI3Ks, binds p101/p84 regulatory subunits which do not have homology to p85 or other proteins, and which permit p110γ to engage with Gβγ subunits downstream of GPCRs. Class I PI3Ks can also engage with small GTPases such as members of the Ras (p110α, p110δ, p110γ) or Cdc42, Rac or Rab5 families (p110β). Unlike PI3Kα and PI3Kδ, PI3Kβ is also activated by Gβγ subunits downstream of GPCRs and appears to require more inputs to become fully activated compared to PI3Kα. *(Insert):* overall domain structure of the p110 catalytic subunits.

Class I PI3Ks phosphorylate the 3-position of the inositol ring of a specific phosphatidylinositol (PtdIns) lipid, namely phosphatidylinositol-(4,5)-bisphosphate (PtdIns(4,5)P_2), converting it to phosphatidylinositol-(3,4,5)-trisphosphate (PtdIns(3,4,5)P_3, or PIP_3). PIP_3 can be converted to PtdIns(3,4)P_2 following dephosphorylation of the 5'-position by the 5-phosphatases SHIP1 and SHIP2. Together, PIP_3 and PtdIns(3,4)P_2 function as second messengers downstream of class I PI3Ks by interacting with 3-phosphoinositide-binding pleckstrin homology (PH) domains found in diverse proteins, including protein kinases (AKT, BTK), adaptor proteins and regulators of small GTPases. The tumour suppressor phosphatase and tensin homolog (PTEN) 3-phosphoinositide phosphatase

dampens class I PI3K signalling, by dephosphorylating PIP_3 and PtdIns(3,4)P_2. PTEN is frequently somatically inactivated in cancer, through a wide range of mechanisms, including loss-of-expression and/or mutation. PTEN inactivation is also the cause of a developmental syndrome known as PTEN Hamartoma Tumour Syndrome (PHTS) in which one gene copy of *PTEN* has been partially or fully inactivated. Individuals with PHTS are predisposed to benign overgrowths, neurodevelopmental abnormalities as well as specific cancers in adulthood.

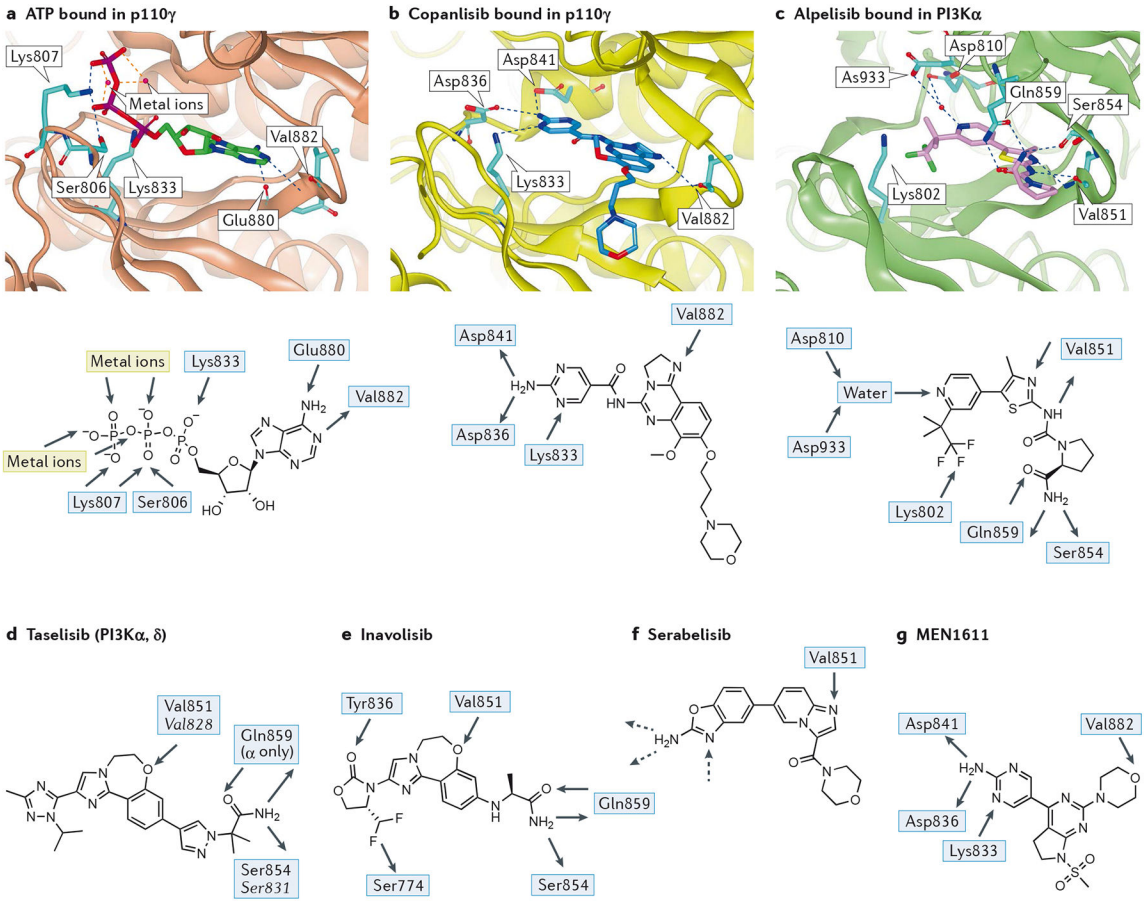

Figure 2 –. Key features of the interaction between PI3Ks and pan- and PI3Kα-selective inhibitors.

The native shape of PI3K enzymes is taken to be that observed by crystallography for ATP-bound p110γ (2a; PDB:1E8X)[18] or the very similar apo forms observed for p110γ (PDB:1E8Y)[18], p110δ (PDB:2WXR)[19] and PI3Kα (in complex with a partial p85α fragment, PBD:2RD0)[20]. Peptides are shown as ribbons with key residues shown in stick representation. Ligands are shown in stick representation. Colour coding of atoms in stick representations - carbon: cyan, oxygen: red; nitrogen: blue; fluorine: green; phosphorus: purple; colour coding of ligands - ATP: green, copanlisib: dark blue, alpelisib: pink, idelalisib: red; hydrogen bonds are shown in blue dashed lines, metal interactions in orange dashed lines.

a) *(Top panel):* **ATP** (carbon atoms bright green) **bound in p110γ** (1E8X)[18]; p110γ shown as brown ribbon with sidechains shown in cyan for residues mentioned in text. The adenine makes an acceptor-donor pair of hydrogen bonds with the NH of hinge Val882 and carbonyl of Glu880 whilst the triphosphate is bound by two metal ions, the terminal ammonium groups of Lys807 and Lys833 and a hydrogen bond from Ser806.*(Lower panel):* 2D representation of the interactions of ATP with the binding pocket of p110γ.

b) *(Top panel)* **copanlisib** (dark blue) **bound in p110γ** (yellow ribbon, 5G2N)[23]. The pendant aminopyrimidine group of copanlisib fits into the affinity pocket and forms H-bonds with Asp836 and Asp841 via the amino group and receives a H-bond from Lys833 to one of

the ring nitrogen atoms. The morpholinopropyl moiety extends towards solvent and does not make any significant interactions; its role in the molecule is mainly as a solubilising group. *(Lower panel):* 2D representation of copanlisib indicating the H-bonds made with PI3Kγ.

c) *(Top panel):* **alpelisib** (pink) **bound in PI3Kα** (green ribbon, 4JPS)[24]. Note the multiple H-bonds: to hinge Val851; involving the primary carboxamide of alpelisib with Gln859 in p110α (Asp862, Lys890, Asn836 in p110β, γ and δ, respectively) and the backbone carbonyl of Ser854; the water-mediated H-bond to the pyridine N from Asp810 and Asp933. The charged terminal amine of Lys802 is close to the CF_3 group. *(Lower panel):* 2D representation of the major interactions of alpelisib in PI3Kα.

d) 2D representation of the PI3Kα/δ inhibitor **taselisib** with H-bonding interactions observed in the crystal with PI3Kα and, in italic, with PI3Kδ. The ether oxygen of taselisib makes the key hinge interaction with both PI3Kα and PI3Kδ. Taselisib has a primary amide that can make the same interactions with p110α as alpelisib[25], but in p110δ a rotation of the side chain places this amide differently, where it can still interact with the backbone carbonyl of Ser831 and places the terminal carbonyl of taselisib towards solvent (PDB:5T8F)[360]. In the affinity pocket, taselisib appears to be capapble of accepting H-bonds from Lys779 (PI3Kδ numbering) to N2 and from a putative water molecule located between Asp787 and Tyr813 (PI3Kδ numbering) to N4.

e) 2D representation of the PI3Kα-selective inhibitor **inavolisib** with H-bonding interactions observed in the crystal with PI3Kα. A carbonyl group in inavolisib accepts an H-bond from Tyr836 in p110α and a difluoromethyl group interacts with the hydroxyl of Ser774 in p110α. Although both of these residues are conserved in all class I PI3K isoforms, the combination of these structural features with a primary amide interacting with the non-conserved Gln859 of p110α results in very high PI3Kα isoform selectivity[25].

f) Structure of the PI3Kα-selective inhibitor **serabelisib**. Although a crystal structure has not been disclosed for this molecule it is probable that the binding mode mimics that of copanlisib (Figure 2b) with the nitrogen of the imidazopyridine accepting a H-bond from the hinge Val851 and the aminobenzoxazole making interactions with the residues in the affinity pocket (hashed arrows).

g) Structure of PI3Kα inhibitor **MEN1611** showing the observed hydrogen bonds in PI3Kγ.

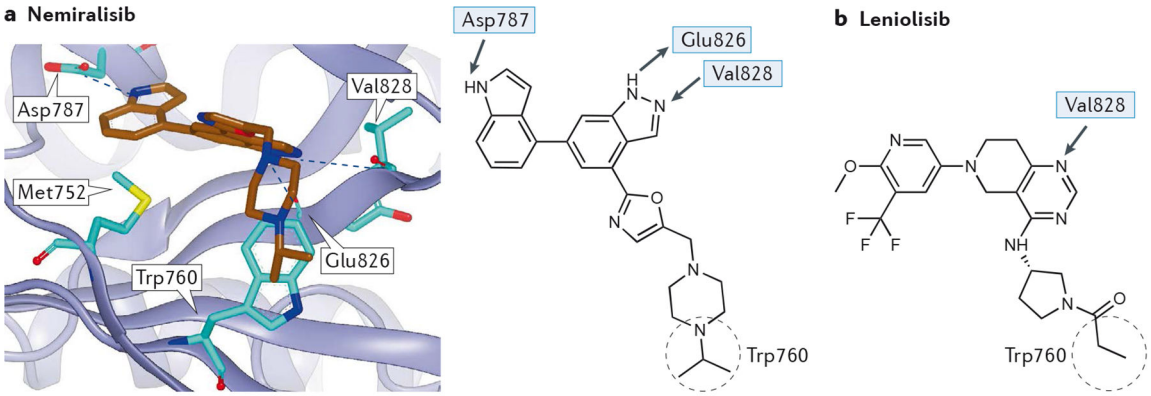

Figure 3 –. Interactions of flat PI3Kδ-selective inhibitors with PI3Kδ

a) *(Upper panel):* **nemiralisib** (brown) **bound in p110δ** (purple ribbon, 5AE8) showing H bonds with the hinge Val828 and adjacent Glu826 plus Asp787. Note that the isopropyl group, though not making any specific interactions, occupies the space above Trp760 in p110δ that is occluded in the other isoforms where the residues corresponding to Thr750 (coloured in green) are larger (Arg770, Lys777, Lys802 in p110α, β and γ, respectively) (*Lower panel):* 2D representation of nemiralisib, with H-bonding interactions and the isopropyl group occupying the tryptophan shelf over Trp760 as observed in the crystal with p110δ.

b) 2D representation of the PI3Kδ-selective inhibitor **leniolisib**, whose quinazoline 1-N accepts an H-bond from Val828 of the hinge. The substituted pyridine occupies the affintiy pocket while the propanoyl pyrrolidine occludes Trp760 giving isoform selectivity in a similar manner to nemiralisib.

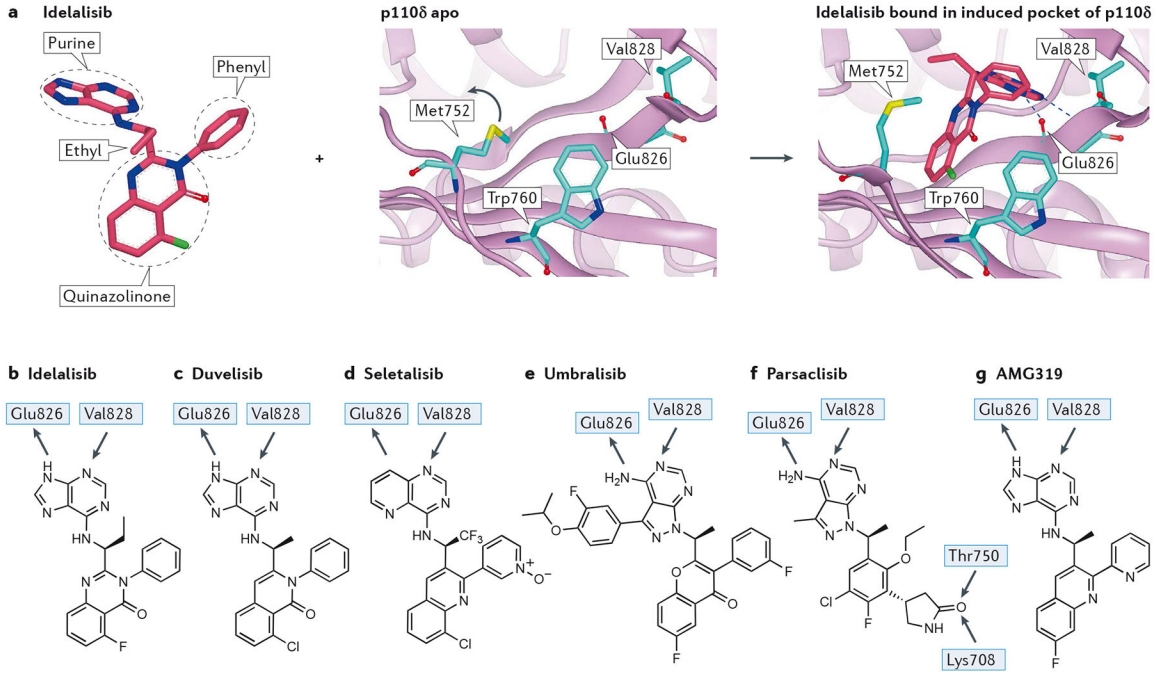

Figure 4 –. Interactions of selected propeller-shaped PI3Kδ-selective inhibitors with PI3Kδ

a) **Inhibitor-induced specificity pocket in PI3K, illustrated by idelalisib binding to p110δ**. *Left panel*: structure of idelalisib from 4XEO drawn to emphasise the propeller shape, thus the three ring systems of the hinge-binding purine, the quinazolinone amd the phenyl are approximately mutually orthogonal in an orientation organised by a combination of the chiral ethyl group and the phenyl ring. *Middle panel:* apo structure of p110δ (2WXR) with Met752 packing against Trp760. The blue arrow indicates the relative motion of Met752 in the flexing of the enzyme in solution that can open up the selectivity pocket. *Right panel:* crystal structure of idelalisib bound in p110δ (4XEO) with the purine making the hinge interaction with the NH of Val828 and the carbonyl of Glu826. The electron deficient quinazolinone ring system fits into the induced selectivity pocket between Met752 and Trp760 and makes a face to edge interaction with the electron rich indole of Trp760.

b) 2D representation of **idelalisib** showing the major interactions with p110δ.

c) 2D representation of the PI3Kγ/δ inhibitor **duvelisib**, with H-bonding interactions observed in the crystal with p110δ. Note the similarity to idelalisib.

d) 2D representation of the PI3Kδ-selective inhibitor **seletalisib**. This is another propeller-shaped PI3Kδ inhibitor, in this case it is probable that the 1 N atom accepts an H-bond from the hinge Val828, with a non-classical H-bond being formed from the CH of the adjacent pyridine ring.

e) Structure of PI3Kδ/CK 1ε inhibitor **umbralisib**. A crystal structure of this has not been published; however, based on the similarity with other propeller inhibitors the structural features can be identified with confidence. The 3-fluoro-4-isopropoxyphenyl ring is similar to substituents in SW13 and SW14 for which crystal structures are known[19]; this occupies the affinity pocket and may be responsible for the high isoform selectivity observed.

f) Structure of the PI3Kδ-selective inhibitor **parsaclisib** with proposed H-bonding interactions based on molecular docking. Note the additional interactions made by the

pendant lactam that accepts two H-bonds from both the hydroxyl of Thr750 (p110δ, Arg770, Lys777, Lys802 in p110α, β and γ, respectively) and the terminal ammonium of Lys708 (p110δ, Gln728, Arg735, Ser760 in p110α, β and γ), respectively; other propeller inhibitors do not have an equivalent group. Despite the multiple structural differences with other PI3Kδ inhibitors, parsaclisib still forms a propeller shape.

g) Structure of PI3Kδ-selective inhibitor **AMG319** showing the hinge interactions with PI3Kδ based on a crystal structure in PI3Kγ.

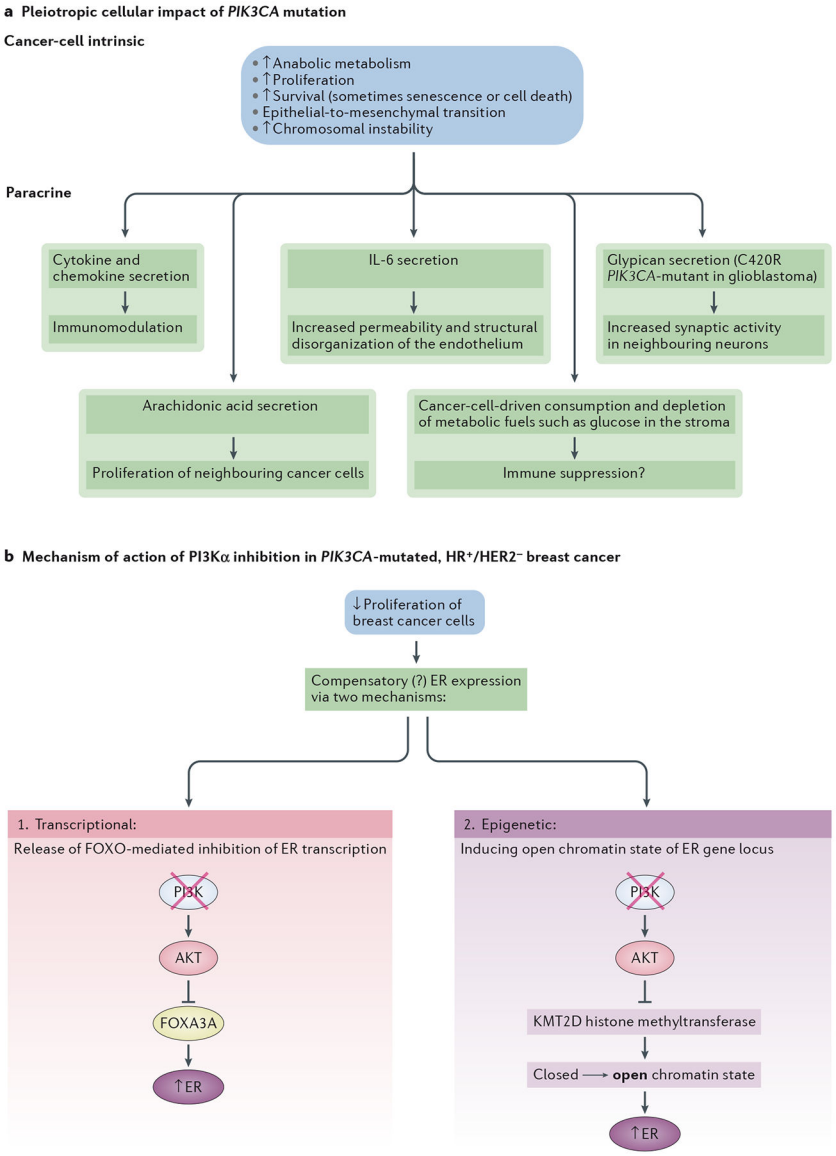

Figure 5 –. Multi-pronged anti-cancer activity of PI3Kα inhibition in solid tumours

a. Pleiotropic effect of *PIK3CA* mutation in solid tumours, inducing both cancer-cell intrinsic and paracrine effects.

b. Proposed mechanisms for the combinatorial anti-tumour effect of anti-PI3Kα and anti-oestrogen therapy in $HR^+/HER2^-$ breast cancer. Anti-proliferation induced by PI3K inhibitionleads to a compensatory expression of the estrogen receptor (ER) and increased dependency on estrogen. The increase in ER transcription can occur via enhanced FOXO3A activity (which is no longer inhibited by active PI3K/Akt)[121] and an epigenetic mechanism through the histone methyltransferase KMT2D which is inhibited upon phosphorylation by AKT[361,362]. Blockade of AKT by PI3Kα inhibition enhances KMT2D activity, leading to a more open chromatin state that facilitates ER-dependent transcription[361]. This epigenetic mechanism can also be transcriptional as it is proposed that KMT2D affects the occupancy of the transcription factor FOXA1, a key regulator of ER binding in breast cancer.

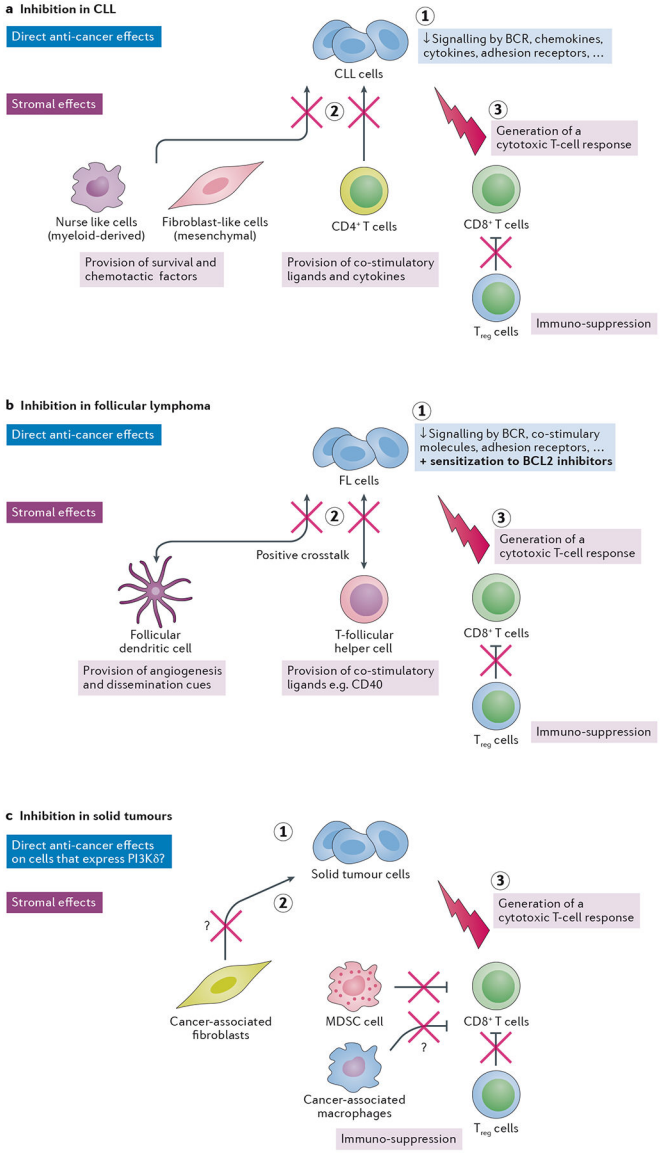

Figure 6 –. Multi-pronged anti-cancer activity of PI3Kδ inhibition in cancer

a. Proposed triple mode-of-action of PI3Kδ inhibition in CLL: (1) a cancer-cell intrinsic impact, with PI3Kδ dampening signalling by the BCR and a range of cytokines, chemokines, co-stimulatory molecules and adhesion receptors; (2) inhibition of stromal cells that support the leukaemic cells, such as myeloid-derived nurse-like cells, mesenchymal fibroblast-like cells and leukaemia-associated T-cells, and (3) a host anti-leukaemia adaptive immune response, as a consequence of dampening of Treg function upon PI3Kδ inhibition. b. Documented effects of PI3Kδ inhibition in FL: (1) a cancer-cell intrinsic impact, with PI3Kδ dampening signalling by the BCR, the CD40/CD40L pathway as well as restoration of FL cell dependence on the BCL2 anti-apoptotic protein, resulting in a predisposition to FL cell death and sensitivity to BLC2 inhibitors; (2) dampening of recruitment of T-follicular helper cells and Treg cells through downmodulation of the CCL22 chemokine; and downregulation of proteins involved in B–T-cell synapses, leading to an inefficient crosstalk

between FL cells and T-follicular helper cells; (3) dampening of follicular dendritic cell-FL interactions related to angiogenesis, cell adhesion and transendothelial migration in FL patients that show a clinical response to PI3Kδ inhibition. (4) a host anti-leukaemia adaptive immune response, as a consequence of dampening of Treg function upon PI3Kδ inhibition. Such a PI3Kδ-inhibition induced immune response has to be formally documented in FL. c. Effect of PI3Kδ inhibition in solid tumours: (1) a cancer cell-intrinsic impact: some solid tumours (such as breast and melanoma) express high levels of PI3Kδ which may provide sensitivity to PI3Kδ inhibition. (2) dampening of the immuno-suppressive effects of MDSCs and macrophages, and dampening of cancer-stimulating fibroblasts and macrophages, and (3) preferential inhibition of Treg cells, allowing a $CD8^+$ T-cell immune response to develop. The question marks in the figure indicate that the role of PI3Kδ in the indicated responses requires further validation, with inhibition of PI3Kγ likely to have a stronger suppressive impact on macrophages than inhibition of PI3Kδ, and blockade of PI3Kα and/or PI3Kβ having a stronger impact than PI3Kδ inhibition on fibroblasts.

Table 1:

Characteristics of clinically-approved PI3K inhibitors to date (March 2020).

Drug/Company	PI3K inhibitors class	Enzyme activities nM (selectivity fold)				Disease indication	Monotherapy or combination	References
		PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ			
Alpelisib/NVP-BYL719/Piqray (Novartis)	PI3Kα inhibitor	4.6	1200 *(260)*	290 *(63)*	250 *(54)*	*PIK3CA*-mutated, hormone receptor-positive (HR^+), human epidermal growth factor receptor-2-negative ($HER2^-$) advanced breast cancer	Combination with the oestrogen receptor (ER) down-regulator fulvestrant	24,44
Idelalisib/CAL-101/GS-1101/Zydelig (Gilead)	PI3Kδ inhibitor	820 *(330)*	570 *(230)*	2.5	89 *(36)*	Chronic lymphocytic leukaemia (CLL), relapsed	Combination with the anti-CD20 antibody rituximab, in patients in whom rituximab alone would be considered appropriate therapy due to other comorbidities	30
						Follicular lymphoma (FL) after at least 2 prior systemic therapies	Monotherapy	
						Small lymphocytic lymphoma (SLL) after at least 2 prior systemic therapies	Monotherapy	
Umbralisib/TGR-1202 (TG Therapeutics)	PI3Kδ inhibitor (also Inhibits CK1ε with IC_{50} 180 nM)	>10000 (>1000)	>10000 (>1000)	6.2	1400 (225)	CLL, FL, MZ lymphoma	2020: fast track FDA approval status in CLL in combination with the anti-CD20 antibody ublituximab; 2021: FDA approval for follicular lymphoma and marginal zone lymphoma	8,253
Duvelisib/IPI-145/Copiktra (Secura Bio)	dual PI3Kγ/δ inhibitor	1600 *(640)*	85 *(34)*	2.5	27 *(11)*	Chronic lymphocytic leukemia (CLL) after at least two prior therapies; follicular lymphoma (FL) after at least two prior systemic therapies; small lymphocytic lymphoma (SLL) after at least 2 prior systemic therapies	Monotherapy	31
Copanlisib/BAY 80-6946/Aliqopa (Bayer)	Pan-PI3K inhibitor	0.5	3.7 *(7)*	0.7 *(1.4)*	6.6 *(13)*	Follicular lymphoma after at least two prior systemic therapies	Monotherapy	23

Table 2:

PI3K inhibitors in clinical development March 2021 and other compounds discussed in the text

Drug names	Company	Enzyme IC_{50}(nM) (selectivity fold)				Disease indications tested in trials	Development phase
		PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ		
Pan PI3K inhibitors							
Buparlisib/NVP-BKM120[21,22]	Novartis → Adlai Nortye	52	166 (3)	116 (2)	262 (5)	head and neck squamous cell carcinoma	Phase III
Pictilisib/GDC-0941[363]	Piramed → Roche/ Genentech	3	33 (11)	3	75 (25)	Breast cancer	Phase I (formerly in Phase II for metastatic breast cancer and non-small cell lung cancer)
PI3Kα inhibitors							
inavolisib/GDC-0077/ RG-6114[25,364-367]	Genentech/Roche	0.034	100 (2900)	12 (360)	18 (540)	breast cancer/other solid tumours	Phase III
serabelisib/INK-1117/ TAK-117/MLN1117/ ART-001/Petra 06[131]	Intellikine → Takeda → Artham (for rare diseases) and Petra Pharma (for oncology)	15	4500 (300)	1900 (130)	14000 (930)	vascular malformations/ solid tumours	Phase Ib/II
MEN1611/CH5132799[133]	Chugai → Menarini	14	120 (8)	500 (36)	36 (2.6)	Breast cancer/coleorectal cancer	Phase Ib/II
CYH-33[368]	Shanghai Institute of Materia Medica → Shanghai HaiHe Biopharma	5.9	600 (100)	79 (13)	225 (38)	Solid tumours	Phase I
PI3Kβ inhibitors							
BL140[43]	Xi'An Jiaotong University School of Medicine	880 (150)	5.7	4200 (145)		N/A	Not in clinical development No data reported for PI3Kγ
SAR260301[369]	Sanofi	1500 (65)	23	470 (20)	>10000 (>4300)	N/A	no further clinical development
GSK2636771[370]	GlaxoSmithKline	>5800 (>1115)	5.2	58 (11)	>126000 (>24,231)	N/A	no further clinical development
AZD8186[371]	AstraZeneca	35 (9)	4	12 (3)	675 (170)	N/A	no further clinical development
PI3Kγ Inhibitors							
eganelisib/IPI-549[37]	Infinity	3200 (200)	3500 (220)	>8400 (>350	16	immuno-oncology	Phase II
AZD3458[41]	AstraZeneca	7900 (11000)	>31000 (>44000)	310 (440)	0.7	N/A	no clinical development

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Drug names	Company	Enzyme IC_{50}(nM) (selectivity fold)				Disease indications tested in trials	Development phase
		PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ		
PI3Kδ inhibitors							
AMG319/ACP-319[372]	Amgen and Cancer Research UK; Amgen → Acerta	33000 (1800)	2700 (150)	18	850 (47)	solid tumours; haemato-oncology	Phase II[235]
nemiralisib/ GSK2269557[27]	GlaxoSmithKline	5000 (39000)	1600 (13000)	0.13	6300 (50000)	airway inflammation e.g. COPD	Inhaled; on hold
leniolisib/CDZ173[373]	Novartis → Pharming	240 (21)	420 (38)	11	2200 (200)	activated PI3Kδ syndrome	Phase II/III
parsaclisib/INCB-50465[33]	Incyte	>20000 (>20000)	>20000 (>20000)	1.1	>10000 (>10000)	haemato-oncology, solid tumours	Phase III
seletalisib/UCB 5857[32]	UCB	3600 (300)	2100 (177)	12	280 (23)	immune-inflammation (eg. Sjögren syndrome)	on hold
zandelisib/PWT-143/ ME-401[254]	Pathway Therapeutics → MEI Pharma	5000 (1000)	210 (42)	5	2100 (420)	haemato-oncology	Phase II
IOA-244[17,249]	Merck AG → iOnctura	19000 (130)	2900 (20)	150	>20000 (>130)	solid tumours	ATP non-competitive; Phase I
linperlisib/YY-20394[231]	Shanghai Yingli Pharmaceutical	1200 (260)	140 (30)	4.6	5200 (1100)	haemato-oncology	Phase II ; Trials planned in solid tumours
CHF-6523[251]	Chiesi	(>454)	(>454)	2.2	(>454)	COPD	Inhaled Phase I
SHC014748[374]	Nanjing Sanhome Pharmaceutical	240 (310)	96	0.77 (125)	101 (130)	Follicular lymphoma, marginal zone lymphoma	Phase II
dezapelisib/ INCB-040093[375]	Incyte	29000 (8500)	3800 (1100)	3.4	2300 (670)	haemato-oncology	Phase II
dual PI3Kγ/δ inhibitors							
tenalisib/RP6530[376]	Rhizen	(>300)	(>100)	25	33 (1.3)	haemato-oncology	Phase II
AZD8154[250]	AstraZeneca	60 (100)	1250 (2000)	0.6	0.8	asthma	Inhaled Phase I
Inhibitors with undisclosed PI3K isoform-inhibitor profiles							
KA2237[377]	Karus Therapeutics	Not disclosed, referred to as a dual PI3Kβ/δ inhibitor				haemato-oncology	Phase I
TQ-B-3525[378] *(a)*	Jiangsu Chia Tai Tianqing Pharmaceutical	Not disclosed, referred to as a dual PI3Kα/β inhibitor				haemato-oncology	Phase II
HMPL-689 *(a)*	Hutchison China MediTech (Chi-Med)	Not disclosed				haemato-oncology	Phase I/II
Indirect PI3Kγ/δ inhibitor: SHIP1 activator							
rosiptor/AQX-1125[260-263]	Aquinox	Not applicable				bladder pain, asthma, COPD	discontinued

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Arrows indicate the trajectory of specific compound series through different commercial entities.

(a) structure and data not disclosed.

Table 3:

Emerging clinical opportunities for PI3K inhibitors

Therapeutic strategy	Therapeutic area	Disease indication	Expected effect of drug
PI3Kα inhibitors	Cancer	Solid tumours, most effective in *PIK3CA*-mutant cancers? (key indications in breast cancer, head and neck cancer and ovarian cancer)s	Direct anti-proliferative effects on cancer cells
			Potentiation of hormone therapy (breast cancer)
			Overcoming anti-HER2 resistance (breast cancer)
			Sensitization to PARP inhibitors or paclitaxel (ovarian and breast cancer)
			Anti-angiogenesis?
			immunomodulation?
	Non-cancer	PROS	Reduction of tissue overgrowth
			Anti-seizure effects
		Obesity and metabolic syndrome	Decrease in adiposity
PI3Kδ inhibitors	Cancer	B-cell malignancies	Direct anti-tumour effects *(anti-proliferative / non-cytotoxic)*
			Interference with B-cell/stroma interaction
		Solid tumours (most effective in ‘immune hot’ tumours)	Activation of host anti-tumour immune response
			Direct anti-tumour effects in PI3Kδ-expressing cancers?
	Non-cancer	APDS	Normalisation of deregulated immune signalling as consequence of PI3K activation
		Auto-immunity/inflammation?	Normalisation of overactive immune signalling
		Diabetic retinopathy?	Dampening of angiogenesis and immunomodulation in endothelial cells
		Infectious diseases such as *Leishmania*	Enhanced innate myeloid cell responses
			Dampened regulatory T and B lymphocyte responses
Pan-PI3K inhibitors	Cancer	B-cell malignancies	Direct anti-tumour effects *(non-cytotoxic)*
			Interference with B-cell/stroma interactions
			Activation of a host anti-tumour immune response?
		Solid tumours	Direct anti-tumour effects
			Interference with how tumour cells modulate their stroma?