PI3K inhibitors are finally coming of age

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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, four PI3K inhibitors have now received regulatory approval – the PI3Kα isoform-selective inhibitor alpelisib for the treatment of breast cancer, and idelalisib, duvelisib and copanlisib which mainly target leukocyte-enriched PI3Kδ in B-cell malignancies. PI3Kα inhibition is most effective in cancers with genetic activation of this isoform, while the anti-leukemic effect of PI3Kδ inhibition is independent of the cancer genomic landscape and instead attributed to dampening of B-cell antigen receptor signalling and tumour-stroma interactions, as well as immunomodulatory effects. This review summarises key discoveries aiding the clinical translation of PI3Kα and PI3Kδ inhibitors, highlighting lessons learned and future opportunities.

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) dampens class I PI3K signalling (**Figure 1**), by dephosphorylating the 3-position in PIP_3 and PtdIns(3,4)P_2. PTEN is frequently somatically inactivated in cancer.

Given its key role in cancer and immunity, the PI3K pathway has been the focus of intensive 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, and 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 review 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. We first summarize the current landscape of PI3K inhibitors and the general principles of isoform-selective PI3K inhibitor development. Reflecting the differing roles of PI3Kα and PI3Kδ, we then summarize their respective biologies, the clinical experience targeting these PI3Ks and emerging opportunities, 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**)[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 (**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[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, with copanlisib representing an optimised development of these chemotypes[21]. The conformation of p110γ bound to copanlisib (PDB: 5G2N)[21] is a little different 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[22] (**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[23]. Inavolisib (GDC-0077) (**Figure 2e**), a further development of the taselisib structure, is significantly more selective and inhibits only PI3Kα. Inavolisib makes more precise interactions in the affinity pocket of p110α along with the PI3Kα favouring carboxamide to give excellent PI3K isoform selectivity[23].

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[24]. Most notably, the exquisitely PI3Kδ-selective inhibitor nemiralisib (GSK2269557, 5AE8)[25] (**Figure 3a**) puts an isopropyl group in this position whilst the less selective leniolisib (**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[26]. Further modifications retained PI3Kγ-selectivity making use only of the difference at Ala885[27].

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[28], duvelisib[29], seletalisb[30] and umbralisib[8] all use this pocket (**Figure 4a-e**).

The highly selective PI3Kδ inhibitor parsaclisib (INCB50465; Incyte; **Figure 4f**)[31] appears to be an optimised 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[32], PI3Kβ/δ[33] and PI3Kβ-favouring[34] 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 (**Figure 5a**) makes use of the same pocket[35].

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 notable PI3K inhibitors

Other 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)[36]. Extending even further from the ATP binding site, Nacht *et al.* developed compounds that form a covalent bond with Cys862 by careful design of an acrylamide substituted inhibitor (3ZIM)[37]. 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[38]. 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[39].

PI3Kβ-selective inhibitors have been harder to find[40], but BL140 (**Figure 5b**), one of the most selective tool compounds reported, has 150-fold and 430-fold selectivity against PI3Kα and PI3Kδ, respectively (no data given for PI3Kγ)[41]. For PI3Kβ inhibitors that have entered clinical development the selectivity, where reported, has been lower, for example TGX-221 (**Figure 5c;** PI3Kβ 5 nM, PI3Kα,γ >100-fold; PI3Kδ 20-fold)[42], AZD8186 (**Figure 5d**; PI3Kβ 4 nM, PI3Kα 9-fold, PI3Kγ 170-fold, PI3Kδ 3-fold)[43] and the more recent GSK2636771 (**Figure 5e**; PI3Kβ 5.2 nM, PI3Kα >1000-fold, PI3Kγ >2000-fold, PI3Kδ 11-fold)[44].

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 the approved compounds inhibit only a single PI3K isoform in the clinical setting. Thus, idelalisib has only 36-fold selectivity for PI3Kδ over PI3Kγ[28]. Duvelisib is closely related to idelalisib (differing only by 4 atoms) and is more potent, but less isoform selective, with 11- and 34-fold selectivity for PI3Kδ over PI3Kγ and PI3Kβ, respectively[29]. 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[21]. While alpelisib exhibits the highest selectivity for PI3Kα, with 50-, 63- and 260-fold selectivity over PI3Kγ, PI3Kδ and PI3Kβ, respectively[22, 45], 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 metabolism (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 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[46, 47]. 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[48] or actin-binding proteins such as gelsolin[49], 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[50].

The generation of mice in which endogenous PI3Kα was rendered inactive[51] and the use of isoform-selective PI3K inhibitors[52], positioned PI3Kα as the main insulin signalling PI3K isoform. Partial PI3Kα inactivation in mice leads to blunted insulin signalling, hyperinsulinaemia and glucose intolerance[51], later found to be the main on-target adverse clinical effects of any inhibitor with activity against PI3Kα[53].

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[54]). Interestingly, normal endometrial epithelium also frequently carries oncogenic *PIK3CA* and *PIK3R1* mutations, the burden of which increases with age and decreases with parity[55].

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[56], which also have the strongest biological impact in experimental cell model systems compared to other *PIK3CA* mutations[57]. It is likely that different *PIK3CA* mutations have distinct biological outputs, as reported in a glioblastoma mouse model[58], but this remains to be investigated in detail. Two activating mutations frequently co-occur in cis on the same *PIK3CA* allele[59-61], the expression of which may render such cells more sensitive to PI3Kα inhibitors compared with cells with single-hotspot *PIK3CA* mutations[59].

Oncogenic mutations in *PIK3CA* mimic and enhance dynamic events in the natural activation process of the auto-inhibited p85-p110 heterodimer[15, 62, 63]. Such processes can also be achieved by mutations in the p85 genes, most commonly in *PIK3R1*[64-66]. *PIK3R1* mutations, common in cancers such as endometrial carcinoma[67], can also activate p110β and p110δ in addition to p110α[66].

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*[68]. 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[69].

In some cancers (including breast[70] and colon[71]), *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 cells of the tumour[72]. 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*[69], in line with findings that cancers often acquire multiple oncogenic hits within the PI3K pathway[73, 74]. Positive selection for oncogenic mutant allele imbalances is frequent in cancer and has also been documented for Ras and other oncogenes[75, 76]. 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[69].

Wild-type *PIK3CA* is frequently amplified in some cancers[54] such as endometrial[77] and lung squamous carcinoma[78], 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[45]. However, in contrast to expression of mutant *PIK3CA*[79, 80], 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[81, 82]. 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*[80]. It is challenging, however, to overexpress p110α protein in cells, most likely because the limiting availability of p85 that are needed to stabilize the labile p110α protein.

Pleiotropic impact of PIK3CA mutation (**Figure 6a**)

In isogenic cancer cell lines, derived by disruption of the wild-type or mutant allele of *PIK3CA*[79, 83], *PIK3CA* mutation has multiple impacts: reduced growth factor dependence yet little effect on cell proliferation under nutrient-rich conditions[79], increased *in vitro* cell migration and invasion[79, 84] and

reduced sensitivity to starvation-induced apoptosis[79]. At the cellular level, the impact of activating *PIK3CA* mutations is context-dependent, and can range from no effect to enhancement of cell cell proliferation to cell senescence[83, 85-87] or even cell death[88] (reviewed in Ref.[89]). Interestingly, in cells with functional p53, *PIK3CA* mutation leads to activation of p53-dependent growth suppression[83].

Accumulating evidence suggests that oncogenic PI3Kα activation supports the emergence of stem cell-like properties[90]. Activating *PIK3CA* mutations also promote invasive properties and epithelial-to-mesenchymal transition (EMT)[81, 84, 91], which is strongly associated with induction of stemness, phenotypic plasticity and, ultimately, resistance to targeted therapy[92-94]. EMT may also facilitate tumour invasion and metastasis[79, 84]. 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[87, 95-98].

Genetic *PIK3CA* activation may also induce and/or allow cells to tolerate chromosomal instability[99], potentially facilitating and/or driving tumour evolution[100].

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 revealed evidence for the expression of PI3K-driven, NF-κB–dependent target-genes enriched in cytokines, chemokines or secreted proteins[82]. Upon overexpression of the Human Epidermal growth factor Receptor 2 (HER2) in these cells, *PIK3CA* mutation leads to induction of a complex secretome that promotes stem cell enrichment, angiogenesis, EMT, altered immune surveillance and vulnerability towards HSP90 inhibition[87]. *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 of surrounding *PIK3CA* WT cells[101].

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[102, 103], resulting in a depletion of metabolic fuels in the stroma and thus contributing to immune suppression[104].

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[58], 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[105].

In conclusion, *PIK3CA* mutation has a diverse biological impact on cancer 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 normally 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].

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 PI3Kα 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[45] 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[45].

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. This leads to hyperglycaemia and a compensatory insulin release from the pancreas which dampens the effect of PI3K inhibition[12].

Clinical development of PI3Kα inhibitors

The approved PI3Kα 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 a single agent 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[89]. 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 showed 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[121]. Mechanistically **(Figure 6b)**, this involves increased ER transcription via enhanced FOXO3A activity[121] and an epigenetic mechanism through the histone methyltransferase KMT2D which is inhibited upon phosphorylation by AKT[122, 123]. Blockade of AKT by PI3Kα inhibition enhances KMT2D activity, leading to a more open chromatin state that facilitates ER-dependent transcription[122]. Primary and acquired resistance to alpelisib in ER+ breast cancer, mediated by persistent FOXM1 expression, may also be regulated through FOXO3A[124].

Other targeted therapies also enhance the efficacy of endocrine therapy, including inhibitors of AKT[125], mTOR or CDK4/6 (reviewed in Ref.[126]), some of which are likely to be contenders of PI3Kα inhibitors in this clinical setting.

The PI3Kα selectivity of alpelisib (**Table 1**)[22] and its pharmacokinetics enabled successful trials in breast cancer, leading to the drug's FDA approval in 2019[127]. 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 (exons 7, 9 and 20), ER-positive, HER2 receptor-negative ($HR^+/HER2^-$) advanced breast cancer who had received prior endocrine therapy. The median PFS was 5 months in the fulvestrant arm and 11 months in patients treated with alpelisib and fulvestrant. 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[128]. The most frequent side-effects with alpelisib are hyperglycaemia, rash and diarrhoea[129, 130], 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 PI3Kα-selective inhibitors in clinical trials (**Table 2**)

Taselisib (Genentech), a highly-potent dual PI3Kα/δ inhibitor[23, 131] progressed to Phase III studies in breast cancer. 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[132], 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. Inavolisib has been claimed to lead to degradation of mutant PI3Kα specifically[133], though details of the mechanism have not been published. Roche have reported accelerated development of inavolisib into phase III trials in breast cancer.

Serabelisib (MLN-1117/TAK-117/INK-1117) (**Figure 2f**) is a selective PI3Kα inhibitor currently in phase 2 trials. Though not particularly potent against PI3Kα, requiring substantial doses, serabelisib has excellent isoform selectivity[134] and favourable pharmacokinetics[135]. The structural basis for the PI3K isoform selectivity of serabelisib has not been described. This compound has been licenced to Petra Pharma who plan to run a phase Ib/II trial in solid tumours with *PIK3CA* or *KRAS* mutations in combination with an sodium-glucose transport protein 2 (SGLT2) inhibitor (NCT04073680), based on a concept published by the Cantley group[12] (see below).

MEN1611 (CH5132799) is a less selective PI3Kα inhibitor with acceptable human pharmacokinetics [136] that is in Phase 1b/2 clinical trials for breast and colorectal cancers[137].

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.

Emerging insights and opportunities

PI3Kα inhibitors in breast cancer

Future efforts in this area will focus on better patient selection, expansion into breast cancer types other than $HR^+/HER2^-$ cancers and combinations other than with hormone therapy. Given that *PIK3CA* mutations are common across the different types of breast cancer, including triple-negative breast cancer (TNBC)[138], there is an interest to clinically explore PI3K inhibition beyond $HR^+/HER2^-$ breast cancer (reviewed in Refs.[139, 140]).

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[59]) or mutant *PIK3CA* gene copy number[69]. Indeed, in a recent clinical study with the AZD5363 AKT inhibitor, homozygosity of the $AKT1^{E17K}$ mutation was associated with an improved therapeutic response[141]. Similar data have been shown for Ras, where cells with multiple copies of mutant Ras are more sensitive to MAP kinase inhibition[142]. Predictive biomarkers beyond *PIK3CA* status could include a transcriptional PI3K pathway activity score[143] or FOXM1 expression[124]. 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[124].

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, as summarized below.

PIK3CA mutation has been implicated in resistance of breast cancer patients to fulvestrant-CDK4/6 inhibitor combination therapy[144, 145]. 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. Conversily, the CDK4 pathway has also been shown to mediate resistance to PI3K

inhibitors in *PIK3CA*-mutant preclinical models[146]. These observations are the basis for trials to evaluate 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[147]. 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[148], the target of the trastuzumab/herceptin anti-HER2 antibody, with PI3K pathway alterations having been associated with resistance to trastuzumab[149]. 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).

PI3Kα inhibitors beyond breast cancer

Additional therapeutic opportunities for PI3Kα inhibitors beyond cancer include PROS, obesity and metabolic syndrome (**BOX 2; Table 3**). However, given its main utility in cancer, the opportunities or PI3Kα inhibitors 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 6b**). 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[150, 151]. Evidence has been presented that this downregulation of BRCA1/2 gene expression is due to ERK-dependent activation of the ETS transcription factor[150, 151]. 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[152].

Around 15% of gastric cancers present a *PIK3CA* mutation, with *PIK3CA* mutations being enriched in EBV-positive subtypes[153]. A phase I/II trial is currently testing the combination of alpelisib with paclitaxel in this molecular subgroup (NCT04526470).

PIK3CA mutation/amplification is found in 21% of head and neck cancers, and in 56% of the HPV^+ subset of head and neck cancers[154]. A study is evaluating the combination between paclitaxel and alpelisib in patients with head and neck cancer (NCT02051751). Combination of radiation with alpelisib[155] or GDC-0032[156] 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].

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[155].

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 (3 times a week) led to an acceptable safety profile and also enabled higher doses and total weekly exposures as compared to once-daily dosing[135]. An intermittent dosing schedule is also being used for the pan-PI3K inhibitor copanlisib, given intravenously on day 1, 8 and 15 of a 28-day cycle[157, 158] 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[158]. 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[159] 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, 159], 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[99], 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 of tumour evolution, 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[100].

Interestingly, pulsatile pan-PI3K inhibition with copanlisib or BAY1082439 in a range of preclinical syngeneic cancer models induces favourable anti-tumour immunomodulatory effects[160, 161]. A key question is whether such effects could also be achieved by PI3Kα-selective inhibitors, as detailed below.

Anti-angiogenic and immunogenic effects of PI3Kα inhibition

Given its ubiquitous expression, PI3Kα inhibition is expected to affect the tumour stroma, including fibroblasts and endothelial cells[162]. PI3Kα blockade can dampen or normalize tumour angiogenesis in preclinical models[163, 164]. This might be achieved at PI3Kα inhibitor doses which do not affect the tumour cells themselves[164], similar as observed with low doses of the RAD001/everolimus mTORC1 inhibitor[165].

Isoform-selective PI3Kα-inhibitors have little or no effect on lymphocytes[134] and other leukocyte types, and are expected to leave white blood cells largely unaffected. It is tempting to speculate that PI3Kα inhibitors might remove tumour-induced metabolic constraints on immune cells (**Figure 6a**) and could be combined with cancer immunotherapy approaches. As mentioned above, pulsatile pan-PI3K inhibition with copanlisib or BAY1082439 in a range of preclinical syngeneic cancer models induces favourable anti-tumour immunomodulatory effects[160]. Simillar data have been reported with the PI3Kα/β/δ inhibitor BAY1082439 in PTEN-null tumour models[161]. Evidence for induction of favourable immune profile changes by AKT inhibitors in breast cancer has also been reported[166].

PI3Kα inhibitors would also dampen the paracrine, potentially tumour-promoting effects of *PIK3CA*-mutant cells discussed above.

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. Indeed, immunomodulation by PI3Kδ inhibition has resulted in adverse effects which have hampered clinical progress of PI3Kδ inhibitors, but have also opened opportunities in cancer immunotherapy.

PI3Kδ in immunity

Preclinical studies using p110δ KO/KI mice and early-generation PI3Kδ inhibitors[167] revealed roles for PI3Kδ in diverse immune functions, suggesting the potential for PI3Kδ inhibitors in autoimmune and inflammatory disorders[5, 6, 168-171], and allowing the development of cell-based assays for PI3Kδ drug development programmes. These include B-cell activation assays[172-174] and anti-IgE-mediated basophil degranulation tests[175], which have also been used to monitor the impact of PI3Kδ inhibition in whole blood assays from patients (as exemplified in Ref.[30]).

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[176]. Such partnership of PI3Kδ extends to PI3Kβ, as illustrated by the cooperation of these PI3Ks in neutrophil activation by immune complexes[177].

Although PI3Kα plays a minor role in lymphocyte signalling[134, 178], it compensates for PI3Kδ inhibition in B-cell development in mice[134, 179] and in human B-cell malignancies[180, 181]. Interestingly, PI3Kβ is expressed at very low levels in B-cells[179].

PI3Kδ mutation in human immune disease

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[182-186]. Bi-allelic loss of *PIK3R1* (p85α) has also been reported and leads to a block in B-cell development[187].

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[188]. Heterozygously-expressed, activating germline mutations in *PIK3CD* are now known to cause the Activated PI3Kδ Syndrome (APDS)[189] primary immunodeficiency, also known as PI3Kδ-activating mutation causing senescent T-cells, lymphadenopathy and immunodeficiency (PASLI)[190 191-193].

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[194, 195]. These mutant p85α proteins preferentially activate p110δ and not p110α or p110β[194, 195], 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[196].

Immune-related defects in APDS patients frequently include lymphadenopathy and sinopulmonary infections, with an increased predisposition to autoimmune and inflammatory

complications, and lymphoma[191, 193]. 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[191, 197, 198].

Several mouse models with APDS mutations have been generated[197-203], 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[172-174].

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*[189], is present at low frequency in diffuse large B-cell lymphoma[204] and T-cell acute lymphoblastic leukemia[205].

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[206-208]. 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 7a,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[209]. 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[210]. 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[211 210]. 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[212] and FL[213] , 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[212, 214, 215] (**Figure 7a**). PI3Kδ inhibition in these PI3Kδ-expressing cells[215] 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 7a**). Treatment of CLL cells *in vitro* with idelalisib abrogates signalling and survival induced by CD40L or TNFα[206]. Treatment of normal T-cells in *vitro* with idelalisib reduces production of IL-6, IL-10 and TNFα[206]. In patients, decreases in circulating cytokines and chemokines produced by both the CLL and stromal cells are observed following initiation of PI3Kδ inhibitors[207, 216]

(**Figure7a**). 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, including 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 7b**). Noteworthy, both TAMs and FDCs express lectins that activate stereotypic mannosylated residues in the BCR of FL cells, leading to autonomous signalling[217]. 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 7b**)[213].

A third facet of the anti-cancer action of PI3Kδ inhibition is a potential host anti-leukaemia immune response (**Figure 7a,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[218-226]. 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[222]. 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 a preferential inhibition of the immunosuppressive regulatory T-cell (Treg) population over the $CD4^+$ T-helper cells and $CD8^+$ T-cells[222] (reviewed in Ref.[227]), leading to a 'rebalancing' of the adaptive immune system towards a $CD8^+$ T-cell response (**Figure 7c**). This preferential inhibition of Treg cells upon PI3Kδ blockade was subsequently confirmed in other studies in mice[218, 220, 221, 224, 225, 228, 229], in *ex vivo* human T-cell subpopulations from healthy donors[230] and in idelalisib-treated CLL patients[230, 231], with patients who experience toxicity displaying a trend towards a lower Treg percentage and a lower Treg:CD4 ratio compared to patients without toxicity[231, 232]. 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[233].

PI3Kδ inhibition also dampens the activity of cancer-promoting myeloid-derived suppressor cells (MDSCs)[222] and cancer-associated macrophages[234], which also reduces the capacity of the latter cells to produce reactive oxygen species that result in the death of Natural Killer cells[235]. 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.[167]). 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[28, 206, 207, 236-240]. 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[216, 241]. 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[242] and combination trials with rituximab, an antibody directed against the CD20 B-cell surface marker[243] (**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 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[244] and airway inflammation[245] (**Table 2**). The most selective PI3Kδ inhibitors to reach clinical trials are parsaclisib (**Figure 4f**) and nemiralisib (**Figure 3a**).

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). A study of parsaclisib in Sjögren's syndrome (NCT03627065) has been completed but the results 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.

Umbralisib (**Figure 4e**) (TGR-1202; TG Therapeutics)[8] has received fast track status in CLL, and has received conditional FDA approval in FL and marginal zone (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[173, 174, 246]. Umbralisib is highly selective for PI3Kδ and also inhibits casein kinase 1ε[247] which is likely to contribute to a different safety profile to that of other PI3Kδ inhibitors, as explained below.

Zandelisib (ME-401; MEI Pharma; **Figure 5f**) exhibits high selectivity for PI3Kδ[248], and is in a phase II registration trial for FL.

Leniolisib (CDZ173; Novartis; now licenced to Pharming) (**Figure 3b**), is in phase III for APDS[249] (NCT02435173/NCT02859727). A dose-finding trial with leniolisib found evidence for immune normalisation and overall patient benefit with no discernible adverse effects[249]. 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. Leniolisib did not show clear efficacy in Sjögren's syndrome (NCT02775916)[250].

AMG319 (**Figure 4g**; Amgen) has been tested in a window-trial in Head and Neck cancer (NCT02540928), with results to be reported.

Seletalisib (UCB-5857) (**Figure 4d**), is another PI3Kδ-selective inhibitor which exhibits good selectivity against PI3Kα and PI3Kβ[30]. Seletalisib has been in phase II clinical trials for Sjögren's syndrome (NCT02610543), but development in this indication appears to have been stopped[251]. In a

proof-of concept study in APDS1 and APDS2 patients, seletalisib demonstrated modest activity and significant side effects (liver injury, colitis, infections)[252]. Such adverse effects were not observed in the APDS patients in the trials with leniolisib mentioned above[249], for reasons that are currently unclear.

The non-ATP competitive PI3Kδ inhibitor IOA-244 (iOnctura; **Figure 5g**), though not particularly potent, has a different binding mode and consequently the potential for very high isoform and kinase selectivity and thus fewer off-target side-effects[17, 253]. 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 inhaled dual PI3Kγδ inhibitor AZD8154 (AstraZeneca; **Figure 5h**) and the inhaled PI3K inhibitor of undisclosed profile CHF6523 (Chiesi; **Figure 5i**)[254]; are being investigated in asthma (NCT04187508) and COPD (NCT04032535), respectively.

In addition, the PI3Kδ inhibitor YY-20394/linperlisib (Shanghai Yingli Pharmaceutical; structure not disclosed) is being investigated in both haematological and solid tumours[255].

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.[256]; **Table 2**).

The phosphatase SHIP1, which is mainly found in leukocytes, 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; **Figure 5j**)[257, 258] is an allosteric activator of SHIP1 that was investigated in phase II clinical trials for asthma[259] and other inflammatory conditions, however all development was stopped after a phase III trial for interstitial cystitis and bladder pain failed[260].

PI3Kδ inhibitor toxicities

Early clinical observations

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 colitis, hepatotoxicity and pneumonitis[231, 261, 262]. The latter were thought to be immune-mediated and found to be responsive to steroids. The pneumonitis and colitis typically occurred late in the course of treatment, in one analysis at a median of 4 and 7 months, respectively[263].

In follow-up trials, idelalisib was used in a front-line setting in treatment-naïve and often younger patients. In this setting of patients with likely more robust immune systems, more severe autoimmune adverse effects arose that were treatment limiting. These included severe autoimmune hepatotoxicity in one study[231], and deaths due to likely drug-related pneumonitis when combined with a SYK inhibitor, in another study[264].

In parallel, Gilead launched a registration program in first and second-line CLL and FL, enrolling a higher proportion of older patients. A combined analysis of these trials demonstrated a higher death rate in the idelalisib arms compared to the control arms and led Gilead to halt these ongoing trials. Most of the deaths on these trials were bacterial infections sometimes in the setting of neutropenia, but cases of cytomegalovirus (CMV) infection and *Pneumocystis* pneumonia were also observed.

From this body of experience, it is clear that adverse effects observed upon PI3Kδ inhibition include bacterial infections (possibly in part secondary to drug-induced neutropenia), opportunistic infections (fungal infections and reactivation of CMV) and inflammatory/autoimmune toxicities including colitis, hepatotoxicity/transaminitis and pneumonitis[231, 261, 262].

Given the complex immune impact of PI3Kδ inhibition, with elements of both immune suppression and activation[233], explaining the adverse effects observed is challenging , yet their range and complexity are not altogether surprising. 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[265]. Retrospectively, it would have been advisable to mandate antimicrobial prophylaxis in idelalisib trials[266]. This is standard procedure when testing immunomodulatory agents in potentially immune-compromised patients but was not mandatory in these trials.

PI3Kδ inhibitor treatment is now considered a much safer treatment option than at the time of the early clinical trials[256]. Antimicrobial prophylaxis as well as CMV monitoring has now been included in the guidelines for clinical use of PI3Kδ inhibitors[266]. 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, either systemic or non-absorbable budesonide for colitis[267].

Recent analysis indicated idelalisib monotherapy to be suitable for heavily-pretreated relapsed/refractory FL patients, given the unmet need in these patients[268]. This conclusion is based on a *post hoc* subgroup analysis of such patients enrolled in an idelalisib monotherapy trial[242] in which the benefits in increased PFS were considered to outweigh the safety concerns in this setting[268].

A 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[269]. 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[270]. It is also possible that the anti-tumour effects of PI3Kδ inhibition will outweigh this potential negative side effect.

Reduced toxicities of newer PI3Kδ inhibitors?

A key question is whether drug chemistries other than idelalisib will result in similar toxicities. Most of the observed immune effects are likely to be on-target, and either result from immune suppression (such as CMV reactivation) or from an overactive immune response in tissue locations exposed to external immunogens (skin, lung, bowel and liver).

There has been speculation that the primary liver metabolite of idelalisib, GS-563117[271], 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[272]. 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[228, 273]. On the other hand, an individual who is null for *PIK3CD* has also been reported to have autoimmune hepatitis[185], 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 7**). 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[262]. Furthermore, in that study, recurrence of transaminitis with resumption of idelalisib could be blocked by concomitant corticosteroids, consistent with an immune-mediated mechanism.

An interesting observation is that the PI3Kδ inhibitor umbralisib (TG Therapeutics), which has shown promising clinical efficacy in CLL, appears to lead to fewer adverse events such as colitis compared to idelalisib and duvelisib[8]. This may relate to the fact that (1) umbralisib, unlike most other inhibitors, is not metabolized through the classical cytochrome P450 (CYP) metabolic pathway [274], and that (2) umbralisib also inhibits casein kinase 1ε (CKε)[247]. Inhibition of CKε on its own *improves* CLL Treg number and function[275]. It is therefore likely that the CKε inhibition by umbralisib protects Tregs from the inhibitory effects of PI3Kδ blockade[275]. This 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.

Improved PI3Kδ inhibitor dosing regimens

Finding tolerable drug dosing regimens is the key challenge to further development of PI3Kδ inhibitors.

Auto-immune and inflammatory diseases likely require lower PI3Kδ inhibitor doses than in cancer, where high doses have been shown to stimulate immune responses. Lower drug doses may still enable the 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[276]. 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[261, 266, 267], with data showing no adverse clinical impact of dose interruptions. Indeed, progression-free survival was fond to be 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[277]. This finding may in part be related to duration on therapy, although it also raises the possibility that these patients with toxicity are also developing an adaptive anti-tumour response (**Figure 7**), as described above.

Companies are now adapting PI3Kδ inhibitor dosing and scheduling regimens, which might also facilitate combination therapies[278]. Trials of parsaclisib in B-cell malignancies, using daily dosing for 9 weeks, followed by once weekly dosing, have lowered the typical toxicity profile of PI3Kδ inhibition and prolonged patient time on-study[273]. Zandelisib/ME-401 (MEI Pharma) was also first investigated on a continuous dosing schedule, with rates of grade 3 immune-mediated adverse events, particularly diarrhoea and rash, reaching 38%. The cumulative incidence was substantially reduced with switching to an intermittent dosing schedule, supported by a 28h plasma half-life and high tissue accumulation of zandelisib/ME-401[279].

Future opportunities

Below, we discuss clinically-advanced opportunities for PI3Kδ inhibitors. Preclinical data suggests additional potential therapeutic opportunities that have yet to be tested in the clinic (**BOX 3**).

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[276]. Topical routes of drug administration have also been explored, for example by inhalation of the PI3Kδ inhibitor nemiralisib (GSK) for inflammatory airway diseases[280]. 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)[281].

Further clinical development in this area 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

Further 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 the leukaemia on chronic BCR signalling which is critically dependent on PI3Kδ, and will 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 has been high in combination therapies that enable deeper remissions and discontinuation of therapy. While some combinations are likely safe, such as with antibodies to CD20 in relapsed CLL[282], others, particularly with chemotherapy, are not without their risks[265], especially in treatment-naïve patients[262]. PI3Kδ inhibition restores the dependence of FL cells on the anti-apoptotic protein BCL2[213], 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 or PI3Kδ inhibitor therapy[274].

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 7c**). The high expression of non-mutated PI3Kδ in some solid tumours such as melanoma or breast[283] 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[234, 284] (reviewed in Ref.[285]).

The immunomodulatory dose of PI3Kδ inhibitors are likely to differ from the maximum-tolerated dose often favoured in oncology, and may also be most effective upon intermittent dosing. Of interest, ntermittent administration of the dual PI3Kα/δ inhibitor AZD8835 induces potent immune-mediated anti-tumour responses in syngeneic solid tumour models in mice[286]. 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[160, 161].

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 (J.B., unpublished results). This contrasts with treatment with checkpoint inhibitors which often results in long-term auto-immunity[287-290]. Moreover, in contrast to checkpoint antibodies, which remain in the circulation for weeks, interruption of PI3Kδ inhibitor dosing allows a 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 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[223, 291]. The latter 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[292, 293], 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[225]. Based on the observation of strong upregulation of the immune checkpoint receptor LAG-3 on the Treg 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[224].

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[226]. 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[220, 221] or oncolytic viruses[294].

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[220, 295-303], 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 maintain 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[125, 141, 304, 305]. Indications for PI3K inhibitors beyond cancer include overgrowth conditions, obesity and metabolic syndrome and 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[164].

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[231, 262].

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. The main utility of PI3Kδ inhibitors may therefore 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 7a,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[256, 306]. 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[256]. An exciting observation is that treatment interruptions upon adverse events (as long as time off therapy was <8%), did not negatively affect clinical impact, and in fact improved overall survival in CLL patients[277]. It is temtpting 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. In addition, PI3Kδ inhibitors are most likely to also be useful in cancer immunotherapy of solid tumours (**Figure 7c**); the outcome of the ongoing trials in this cancer context are eagerly awaited.

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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[307, 308]. Similarly, treatment with alpelisib of a patient with a *PIK3CA* mutant breast cancer led to recurrent *PTEN* loss in different metastases[309]. 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[310]. 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[45, 311]. Some of these cell lines have been shown to be instead sensitive to the antiproliferative effect of PI3Kβ inhibitors[311-313], although this correlation is not universal[314] (reviewed in Refs.[315, 316]).

A range of resistance mechanisms linked to alterations in PI3K activities or PTEN themselves have also been reported, including amplification of Myc or eIF4E[317], activation of the SGK Ser/Thr kinases (which are highly related to AKT)[318, 319], activation of cyclin-dependent kinases CDK4/6[146] and persistent expression of FOXM1[124]. One study identified 63 putative alpelisib resistance genes, including activation of the PIM Ser/Thr kinases[320]. 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[321]. 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[322, 323]. 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[324]. 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* culture co-culture system of FL patient leukaemic cells mixed with FDCs from normal tonsils treated or not with idelalisib, Serrat *et al.*[213] 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[213].

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)[325] and in disorders belonging to the *PIK3CA*-related overgrowth spectrum (PROS; reviewed in Ref.[326]). The lack of cancer predisposition in these conditions illustrates the context-dependent impact of genetic PI3Kα activation[89].

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[326]. 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[326] (**Figure 6a**). 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[327]. 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[58], endothelial cells[105] and wild-type cancer cells[101]. Paracrine effects upon loss of PTEN expression have also been reported[328].

Treatment of PROS patients with low doses of alpelisib as part of a compassionate use program has shown a promising clinical impact (Novartis; NCT04085653)[329]. Treated patients experienced negligible side effects[329], even upon exposure of some patients to drug for up to 3.5 years (Guillaume Canaud, Paris; *personal communication*). 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[330].

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 answer these questions (NCT04589650/NCT04085653).

Obesity and metabolic syndrome

While heterozygous genetic PI3Kα inactivation in mice leads to adverse metabolic effects at young age[51], such chronic partial PI3Kα inactivation protects older mice from age-related reduction in insulin sensitivity, glucose tolerance and fat accumulation[331]. Chronic partial pharmacological PI3Kα inactivation did also not lead to major toxicities or side effects in mice[332]. PI3K inhibitors also reduce obesity in mice and monkeys[333, 334], attributed to an increased energy expenditure as a consequence of activation of thermogenesis in brown adipocytes[335] and increased oxidative phosphorylation together with reduced anaerobic glycolysis[336]. Upregulation of mitochondrial activity in mouse adipocytes (as well as in *Drosophila* fat bodies)[337] and potentiation of β-adrenergic/cAMP signalling in these cells that leads to increased catecholamine-induced energy expenditure[338], 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[339]. 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[283], 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[340, 341]. 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[342-344] and schizophrenia[341, 345], angiogenesis and immunomodulation in endothelial cells (including in pathological retinal angiogenesis[346, 347] and inflammatory/immunomodulatory functions in fibroblast-like cells such as synoviocytes in arthritic joints[340, 348-351]. PI3Kδ inhibition could also have an antitumour effect by suppressing tumour-promoting PI3Kδ-expressing fibroblast-like cells, namely mesenchymal fibroblast-like cells in CLL[214, 215] and cancer-associated fibroblasts in breast cancer[351] (**Figure 7**).

Figure Legends

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)[21]. 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)[22]. 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[23], 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)[352]. 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[23].

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 (light blue 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 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; based, however, 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 so 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 – Other PI3K pathway inhibitors discussed in the text

For all of these (except IOA-244 and AQX-1125) the identified or probable hinge binding group is drawn at the top.

a) PI3Kγ selective inhibitor eganelisib/IPI-549
b) PI3Kβ selective inhibitor BL140
c) PI3Kβ selective inhibitor TGX-221
d) PI3Kβ selective inhibitor AZD8186
e) PI3Kβ selective inhibitor GSK2636771
f) PI3Kδ selective inhibitor zandelisib
g) Non-ATP competitive PI3Kδ inhibitor IOA-244
h) Inhaled dual PI3Kγδ inhibitor AZD8154
i) Putative structure for inhaled PI3K inhibitor CHF6523[254]
j) SHIP1 activator AQX-1125

Figure 6 – 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 inhibition induces leads 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[122, 123]. Blockade of AKT by PI3Kα inhibition enhances KMT2D activity, leading to a more open chromatin state that facilitates ER-dependent transcription[122]. This epigenetic mechanism can also be transcriptional

as it us propose that KMT2D affects the occupancy of the transcription factor FOXA1, a key regulator of ER binding in breast cancer.

Figure 7 – 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. Such a PI3Kδ-inhibition induced anti-cancer immune response has to be formally documented in FL.
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 an 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 cancer-stimuting macrophages, and (3) preferential inhibition of Treg cells, allowing a $CD8^+$ T-cell immune response to develop.

Table 1: Characteristics of clinically-approved PI3K inhibitors to date (November 2020).

Drug	Enzyme activities nM *(selectivity fold)*				Disease indication	Monotherapy or combination
	PI3Kα	**PI3Kβ**	**PI3Kδ**	**PI3Kγ**		
idelalisib[28]	820 *(330)*	570 *(230)*	2.5	89 *(36)*	1. chronic lymphocytic leukaemia (CLL), relapsed 2. follicular lymphoma (FL) after at least 2 prior systemic therapies 3. small lymphocytic lymphoma (SLL) after at least 2 prior systemic therapies	1. combination with the anti-CD20 antibody rituximab, in patients in whom rituximab alone would be considered appropriate therapy due to other comorbidities 2. monotherapy 3. monotherapy
duvelisib[29]	1600 *(640)*	85 *(34)*	2.5	27 *(11)*	1. chronic lymphocytic leukemia (CLL) after at least two prior therapies 2. follicular lymphoma (FL) after at least two prior systemic therapies 3. small lymphocytic lymphoma (SLL) after at least 2 prior systemic therapies	monotherapy for all
copanlisib[21]	0.5	3.7 *(7)*	0.7 *(1.4)*	6.6 *(13)*	Follicular lymphoma after at least two prior systemic therapies	monotherapy
alpelisib[22, 45]	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

Table 2: Key drug clinical development programmes with PI3K inhibitors

Drug names	**Company**	**Enzyme IC_{50} (nM) *(selectivity fold)***				**Disease indications tested in trials**	**Comments**
		PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ		
Pan-PI3K inhibitor							
copanlisib/BAY 80-6946/Aliqopa[21]	Bayer	0.5	3.7 *(7)*	0.7 *(1.4)*	6.6 *(13)*	haemato-oncology/solid tumours	Approved
PI3Kα inhibitors							
alpelisib/NVP-BYL719/Piqray[22, 45]	Novartis	4.6	1200 *(260)*	290 *(63)*	250 *(54)*	solid tumours/PROS	Approved
inavolisib/GDC-0077/RG-6114[23, 353-356]	Genentech/Roche	0.034	100 (2900)	12 (360)	18 (540)	breast cancer/other solid tumours	Phase III
serabelisib[134]/INK-1117/TAK-117/MLN1117/Petra 06	Intellikine → Takeda → Petra Pharma	15	4500 (300)	1900 (130)	14000 (930)	solid tumours	Phase Ib/II
MEN1611 (CH5132799)[136]	Chugai → Menarini	14	120 (8)	500 (36)	36 (2.6)	Breast cancer/coleorectal cancer	Phase Ib/II
PI3Kβ inhibitors							
SAR26030[357]	Sanofi	1500 (65)	23	470 (20)	>10000 (>4300)	no further clinical development	
GSK2636771[44]	GlaxoSmithKline	>5800 (>1115)	5.2	58 (11)	>126000 (>24,231)	no further clinical development	
AZD8186[43]	AstraZeneca	35 (9)	4	12 (3)	675 (170)	no further clinical development	
PI3Kγ Inhibitors							
eganelisib/IPI-549[35]	Infinity	3200 (200)	3500 (220)	>8400 (>350	16	immune-oncology	Phase II
AZD3458[39]	AstraZeneca	7900 (11000)	>31000 (>44000)	310 (440)	0.7	no clinical development	
PI3Kδ inhibitors							
idelalisib/CAL-101/GS-1101/Zydelig[28]	ICOS → Calistoga → Gilead	820 *(330)*	570 *(230)*	2.5	89 *(36)*	haemato-oncology	Approved
umbralisib/TGR-1202[8, 247]	TG Therapeutics	>10000 (>1000)	>10000 (>1000)	6.2	1400 (225)	haemato-oncology	- 2020: fast track FDA approval status in CLL, - 2021: FDA approval for follicular lymphoma and marginal zone lymphoma - also Inhibits CK1ε with IC_{50} 180 nM

GS-9901[358]	Gilead	750 (750)	100 (100)	1	190 (190)	haemato-oncology	discontinued
AMG319/ACP-319[359]	Amgen and CRUK; Amgen→ Acerta	33000 (1800)	2700 (150)	18	850 (47)	solid tumours; haemato-oncology	Phase II
nemiralisib/GSK2269557[25]	GlaxoSmithKline	5000 (39000)	1600 (13000)	0.13	6300 (50000)	airway inflammation e.g. COPD	inhaled PI3Kδ inhibitor – on hold
leniolisib/CDZ173[360]	Novartis → Pharming	240 (21)	420 (38)	11	2200 (200)	activated PI3Kδ syndrome	Phase II/III
parsaclisib/INCB-50465[31]	Incyte	>20000 (>20000)	>20000 (>20000)	1.1	>10000 (>10000)	haemato-oncology, solid tumours	Phase III
seletalisib/UCB 5857[30]	UCB	3600 (300)	2100 (177)	12	280 (23)	immune-inflammation (eg. Sjögren syndrome)	on hold
zandelisib/PWT-143/ME-401[248]	Pathway Therapeutics → MEI Pharma	5000 (1000)	210 (42)	5	2100 (420)	haemato-oncology	Phase II
IOA-244[17, 253]	Merck AG → iOnctura	19000 (130)	2900 (20)	150	>20000 (>130)	solid tumours	ATP non-competitive
linperlisib/YY-20394[229, 255, 361]	Shanghai Yingli Pharmaceutical	1200 (260)	140 (30)	4.6	5200 (1100)	haemato-oncology	Trials planned in solid tumours (NCT04049929)
CHF-6523[254]	Chiesi	(>454)	(>454)	2.2	(>454)	COPD	Inhaled Phase I
dual PI3Kγ/δ inhibitors							
duvelisib/IPI-145/Copiktra[29]	Intellikine → Infinity → Verastem → Secura Bio	1600 (640)	85 (34)	2.5	27 (11)	haemato-oncology	Approved
tenalisib/RP6530[362]	Rhizen	(>300)	(>100)	25	33 (1.3)	haemato-oncology	Phase II
AZD8154	AstraZeneca	60 (100)	1250 (2000)	0.6	0.8	asthma	Inhaled Phase I
Inhibitors with undisclosed PI3K isoform-inhibitor profiles							
KA2237[363]	Karus Therapeutics	Not disclosed, referred to as a dual PI3Kβ/δ inhibitor				haemato-oncology	Phase I
HMPL-689 [(a)]	Hutchison China MediTech (Chi-Med)	Not disclosed				haemato-oncology	Phase I/II
Indirect PI3Kγ/δ inhibitor: SHIP1 activator							
rosiptor/AQX-1125/rosiptor[257-260]	Aquinox	Not applicable				bladder pain, asthma, COPD	discontinued

Arrows indicate the trajectory of specific compound series through different commercial entities.
[(a)] structure and data not disclosed.

Table 3: Clinical opportunities for approved PI3K inhibitors

	PI3Kα inhibitors		PI3Kδ inhibitors		pan-PI3K inh (copanlisi	
	Disease indication	**Expected effect of drug**	**Disease indication**	**Expected effect of drug**	**Disease indication**	**eff**
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?	B-cell malignancies	• Direct anti-tumour effects (anti-proliferative / non-cytotoxic) • Interference with B-cell/stroma interaction • Activation of host anti-tumour immune response?	B-cell malignancies	• D • I • A
			Solid tumours (most effective in 'immune hot' tumours)	• Activation of host anti-tumour immune response • Direct anti-tumour effects in PI3Kδ-expressing cancers?	Solid tumours	• D • I • P
Non-cancer	PROS	• reduction of tissue overgrowth	APDS	• Normalisation of overactive immune signalling		

	Obesity and metabolic syndrome	• decrease in adiposity	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	

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Figure 1

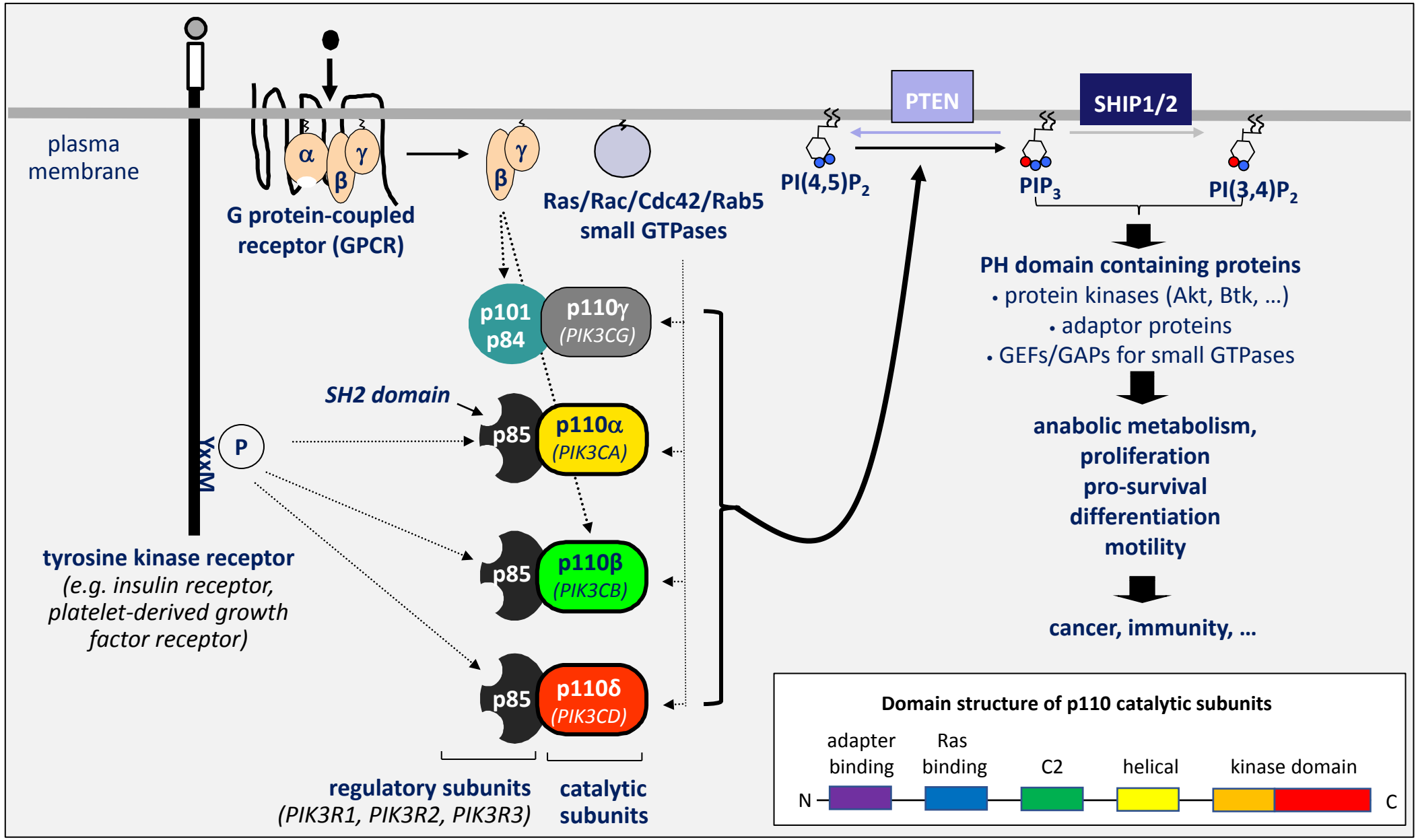
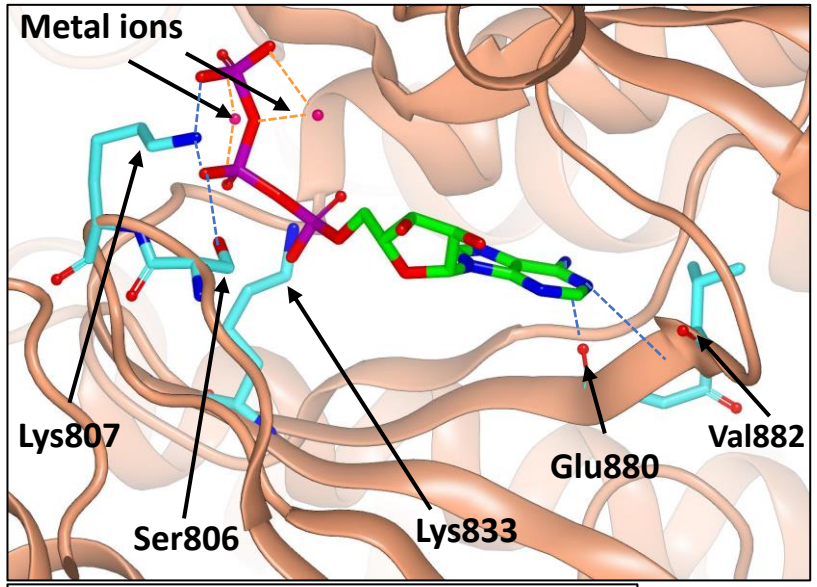
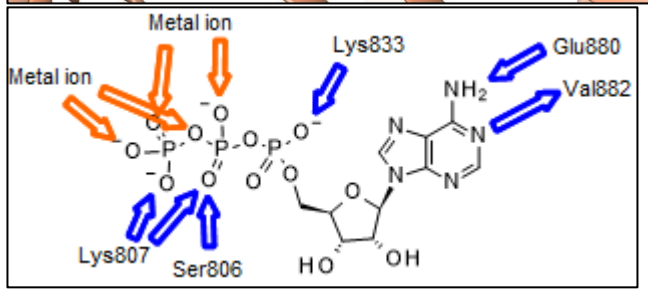

plasma membrane
α
β
γ
G protein-coupled receptor (GPCR)
β
γ
Ras/Rac/Cdc42/Rab5 small GTPases
PTEN
SHIP1/2
PI(4,5)P2
PIP3
PI(3,4)P2
PH domain containing proteins
• protein kinases (Akt, Btk, …)
• adaptor proteins
• GEFs/GAPs for small GTPases
anabolic metabolism,
proliferation
pro-survival
differentiation
motility
cancer, immunity, …
p101
p84
p110γ
(PIK3CG)
SH2 domain
p85
p110α
(PIK3CA)
P
YxxM
p85
p110β
(PIK3CB)
p85
p110δ
(PIK3CD)
tyrosine kinase receptor
(e.g. insulin receptor, platelet-derived growth factor receptor)
regulatory subunits
(PIK3R1, PIK3R2, PIK3R3)
catalytic subunits
Domain structure of p110 catalytic subunits
adapter binding
Ras binding
C2
helical
kinase domain
N
C

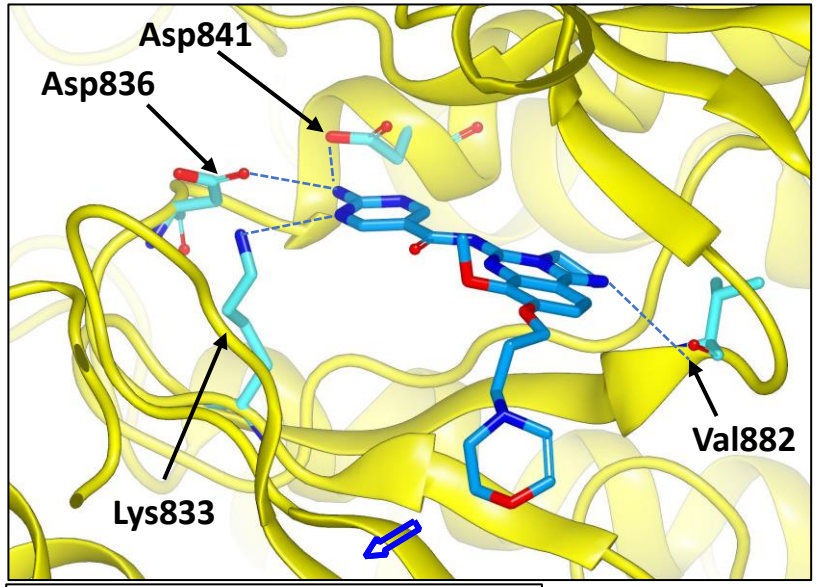
Figure 2

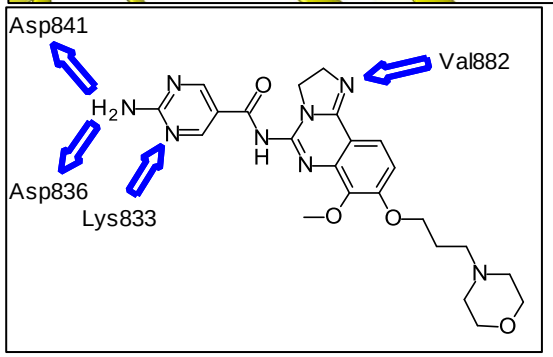
a. ATP bound in p110γ



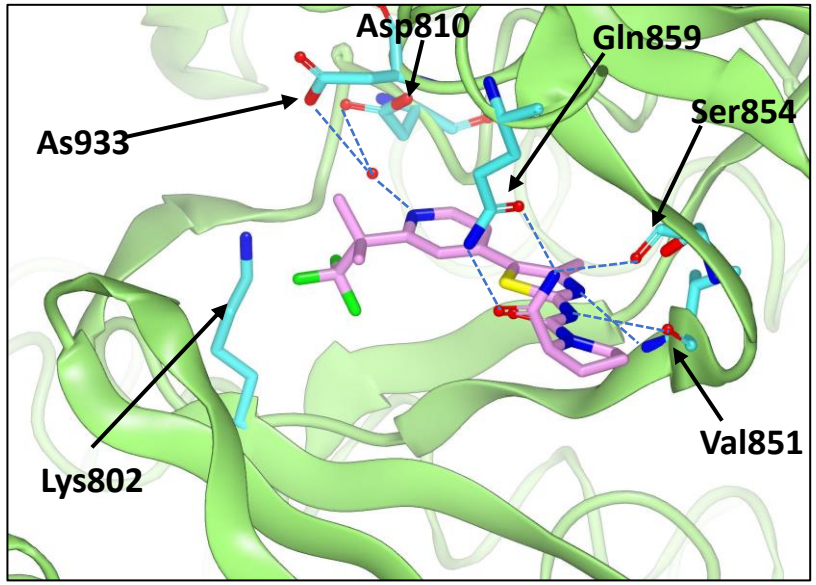


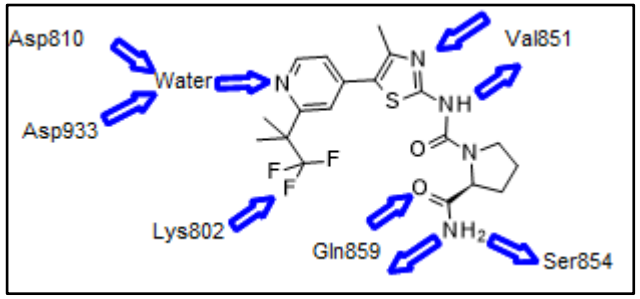
b. copanlisib bound in p110γ



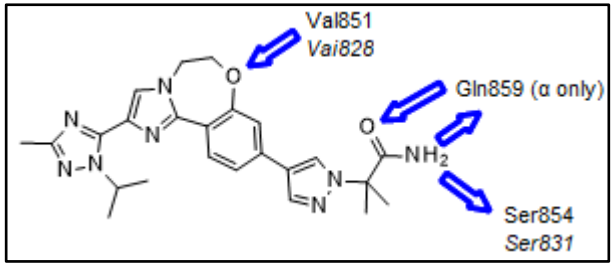


c. alpelisib bound in PI3Kα

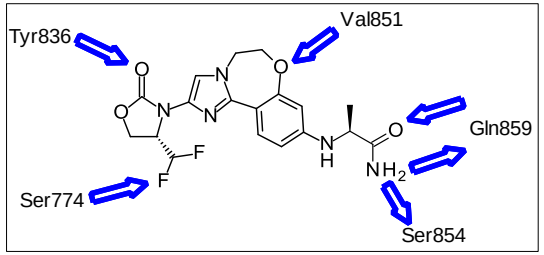




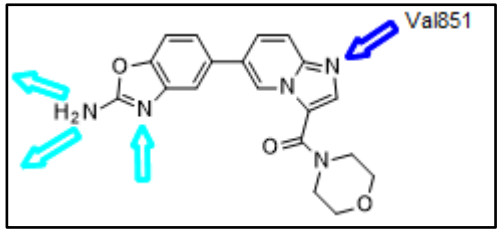
d. taselisib (PI3Kα, *δ*)



e. inavolisib



f. serabelisib



g. MEN1611

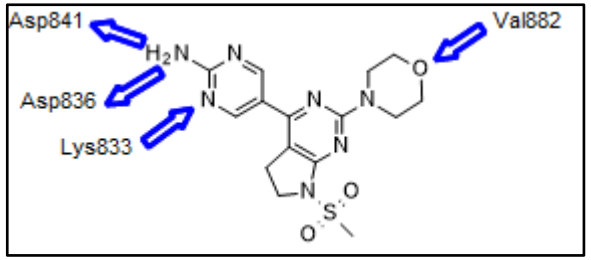
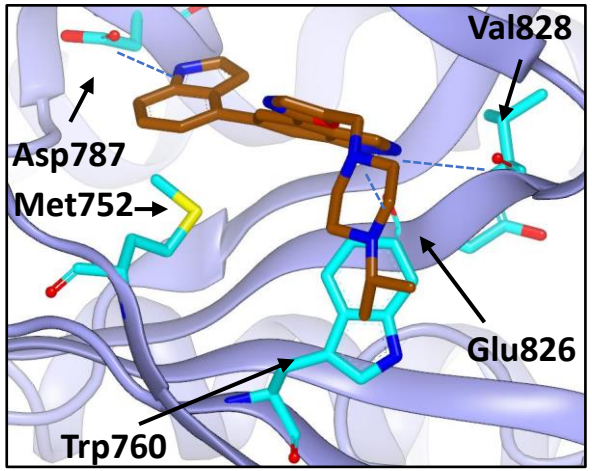
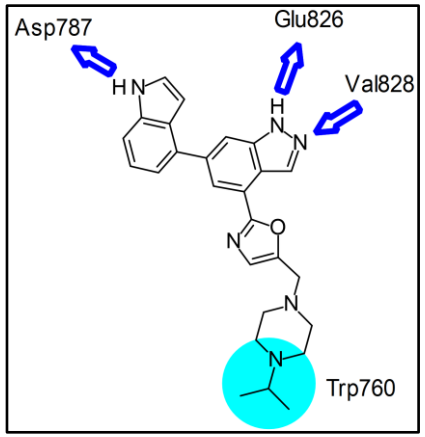


Figure 3

a. nemiralisib





b. leniolisib

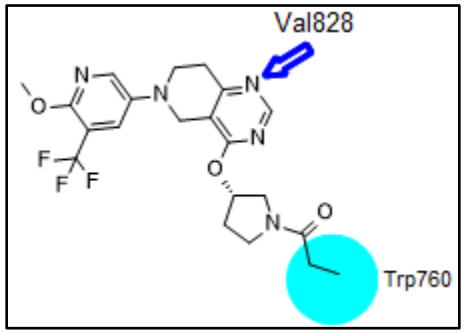


Figure 4

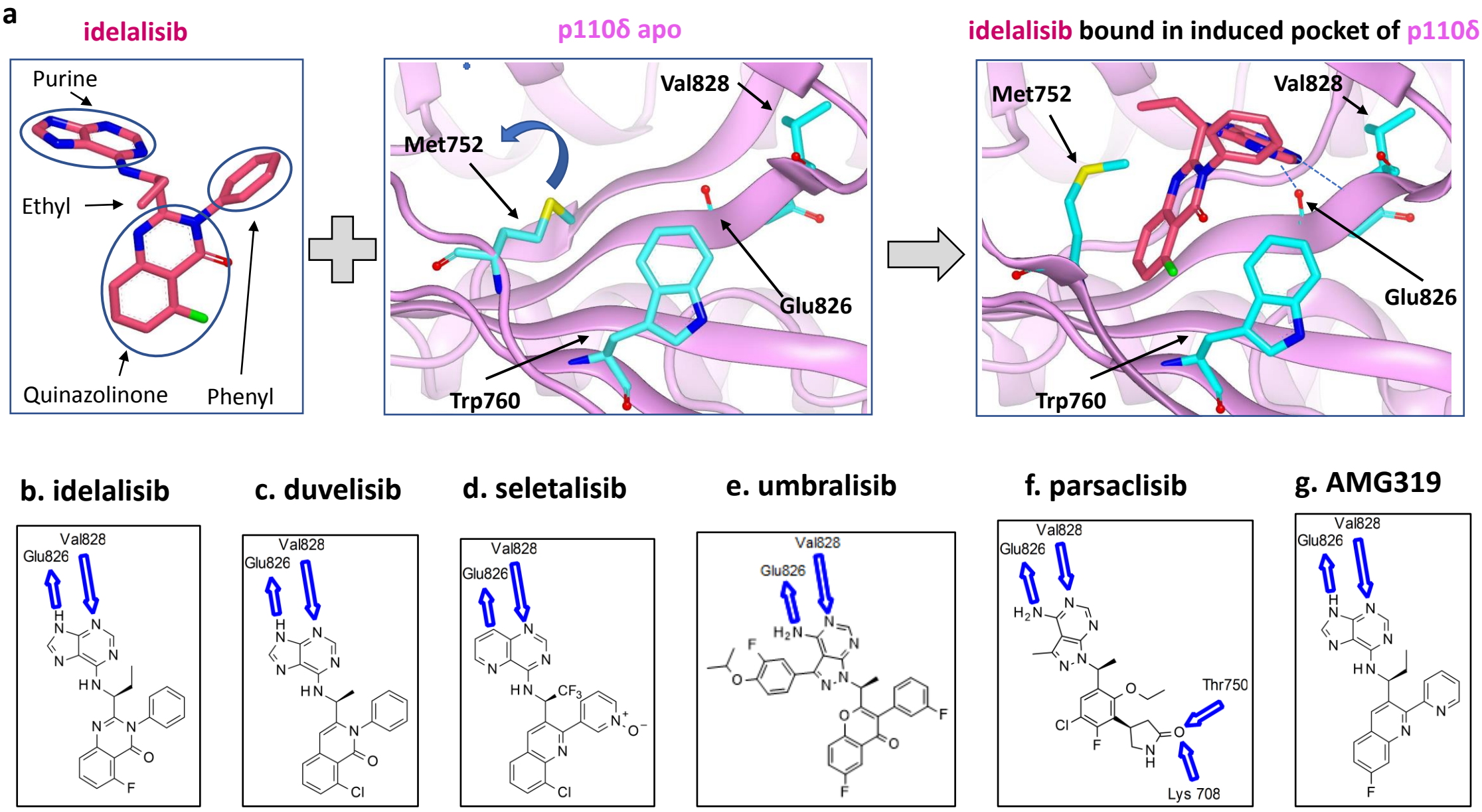
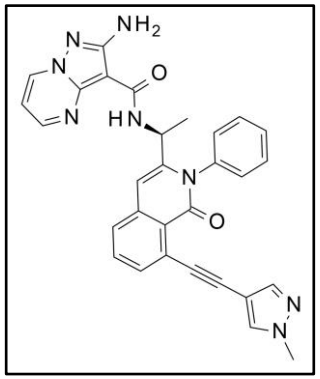
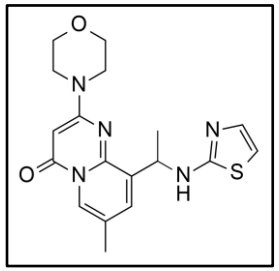

a
idelalisib
p110δ apo
idelalisib bound in induced pocket of p110δ
Purine
Ethyl
Quinazolinone
Phenyl
Val828
Met752
Glu826
Trp760
b. idelalisib
c. duvelisib
d. seletalisib
e. umbralisib
f. parsaclisib
g. AMG319
Thr750
Lys 708

Figure 5

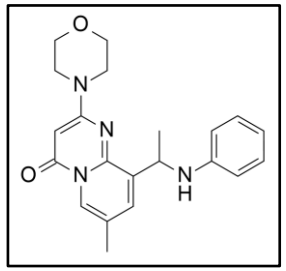
a. IPI-549



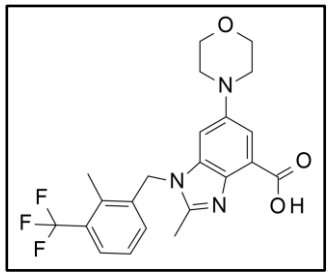
b. BL140



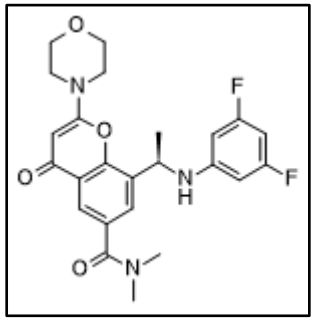
c. TGX-221



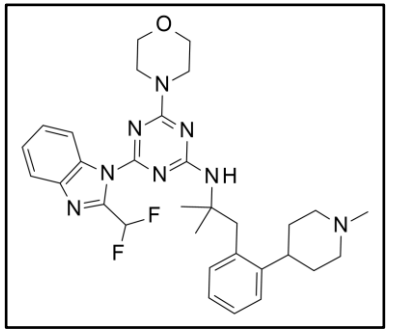
d. GSK2636771



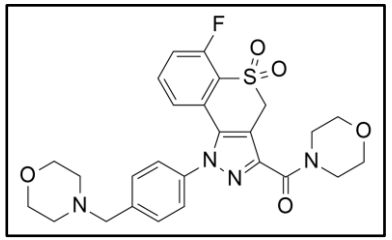
e. AZD8186



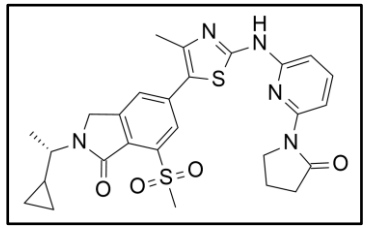
f. zandelisib



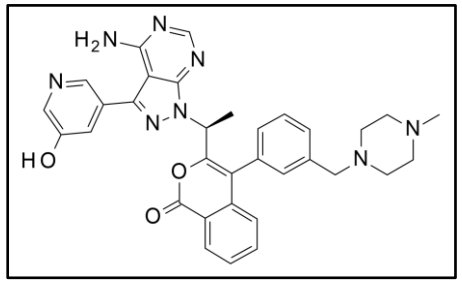
f. IOA-244



g. AZD8154



h. CHF6523



i. AQX-1125

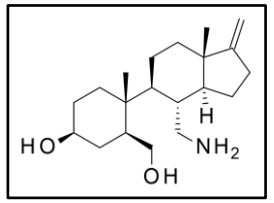
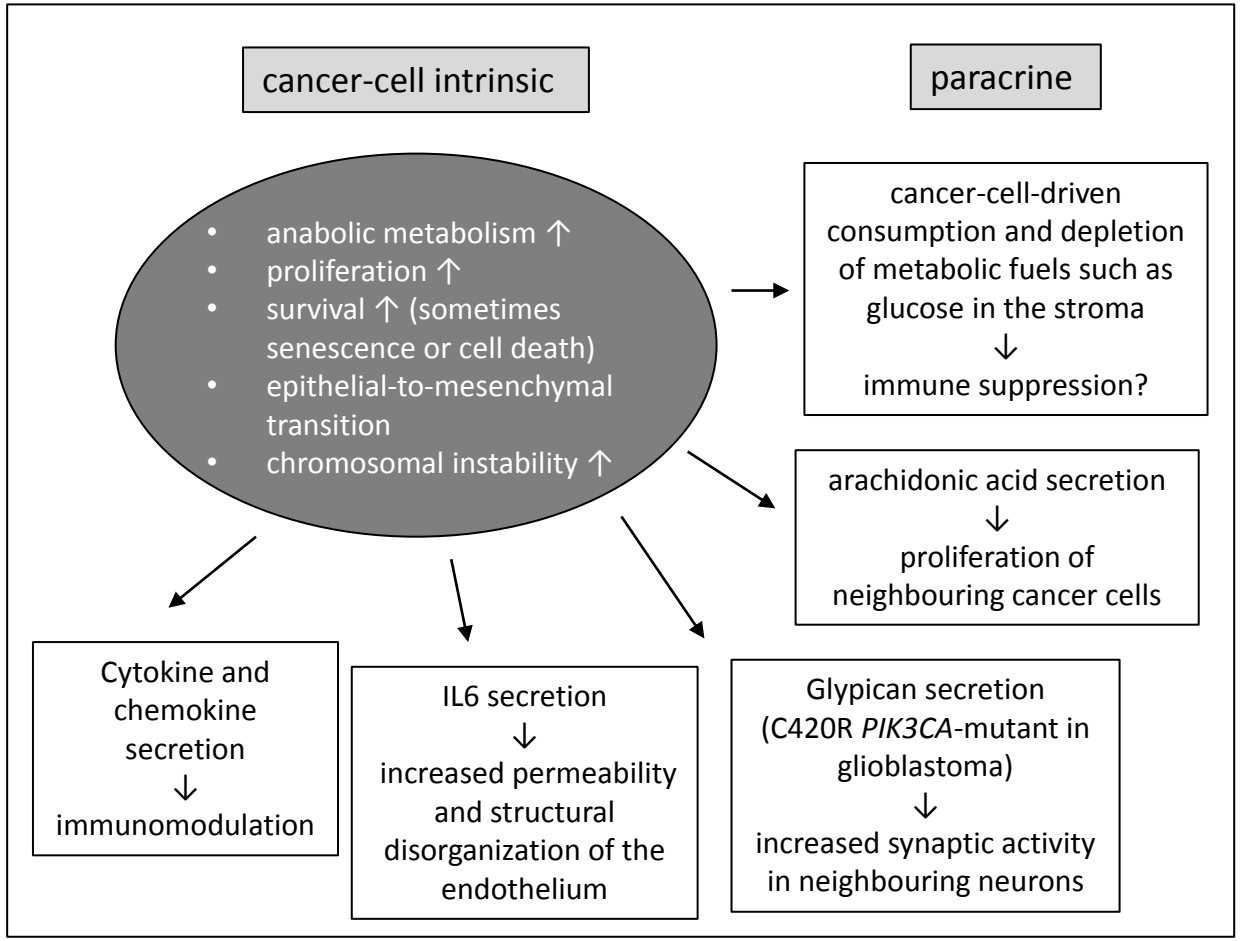


Figure 6.

a. Pleiotropic cellular impact of *PIK3CA* mutation



b. Mechanism of action of PI3Kα inhibition in *PIK3CA*-mutated, HR$^+$/HER2$^-$ breast cancer

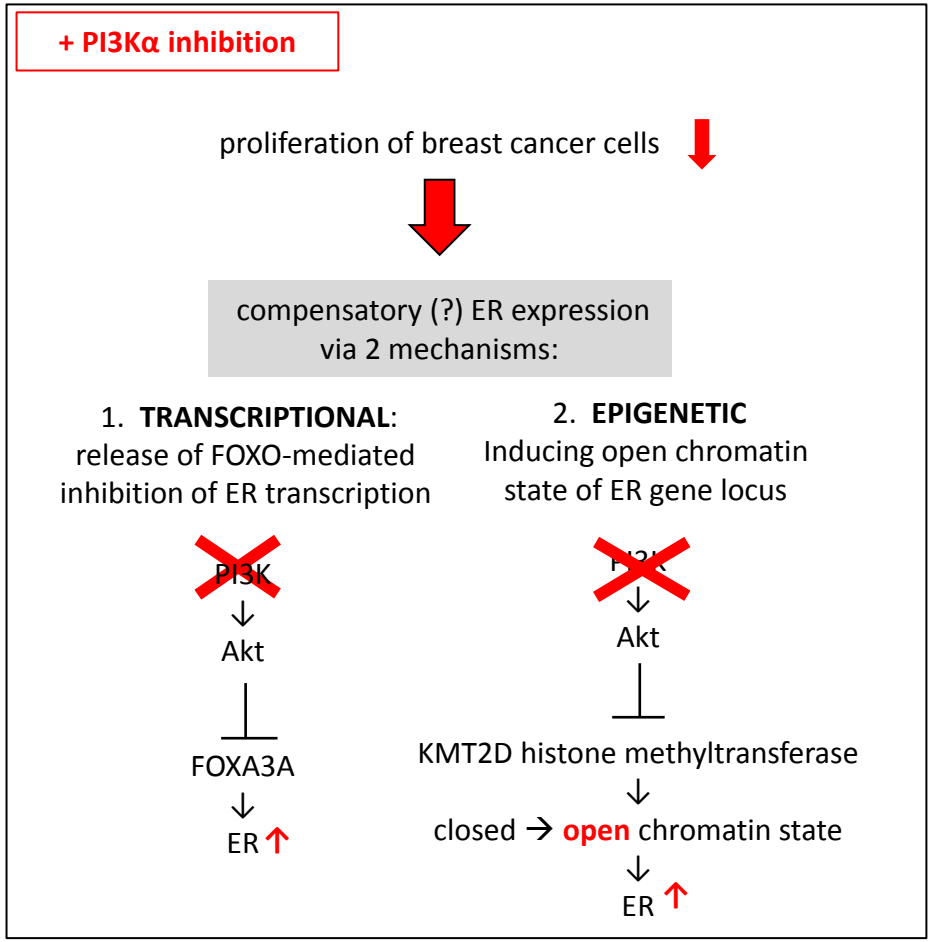


Figure 7: Anti-tumour mechanisms of PI3Kδ inhibition
a. in CLL

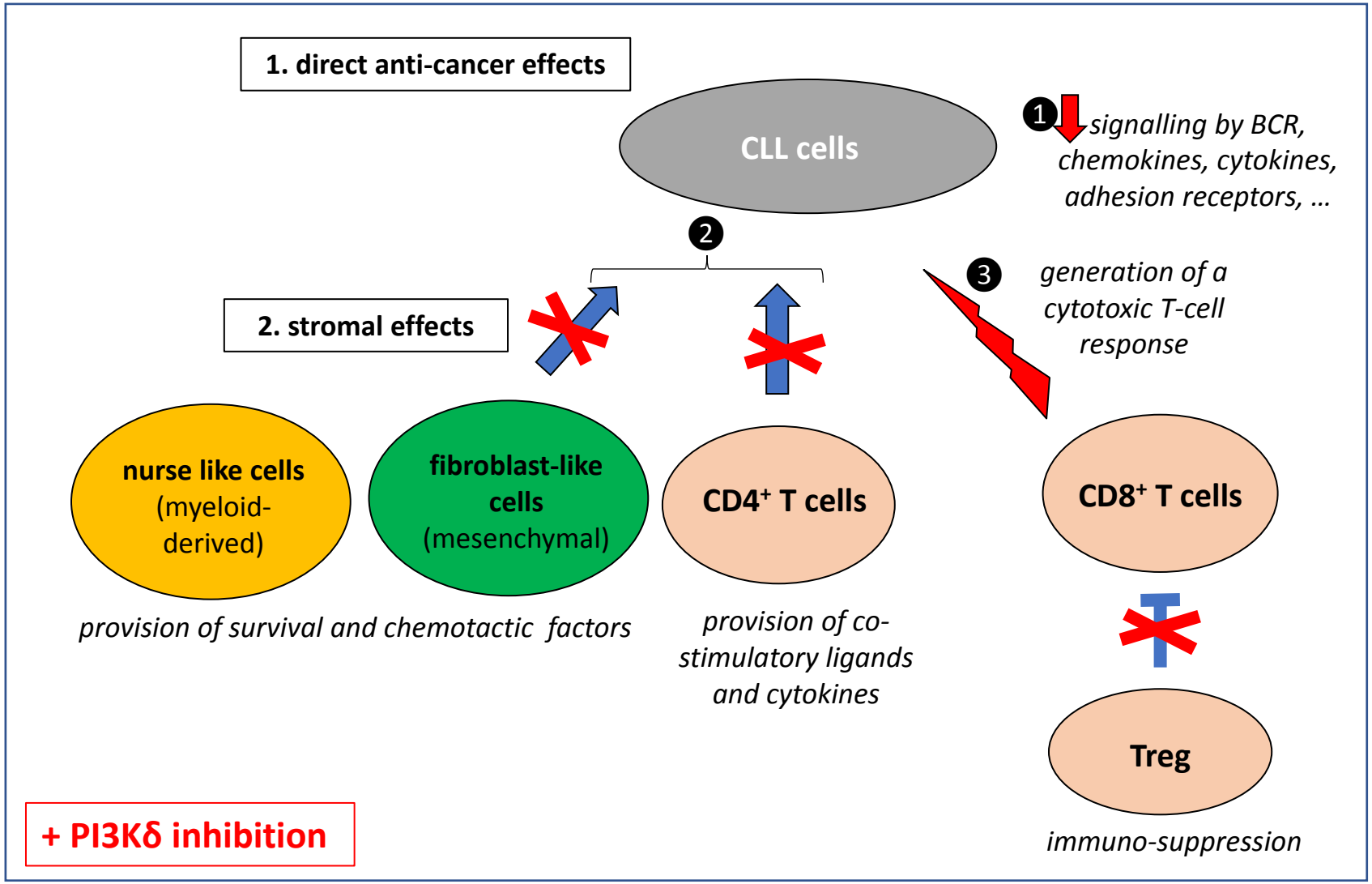


Figure 7: Anti-tumour mechanisms of PI3Kδ inhibition

b. in Follicular Lymphoma

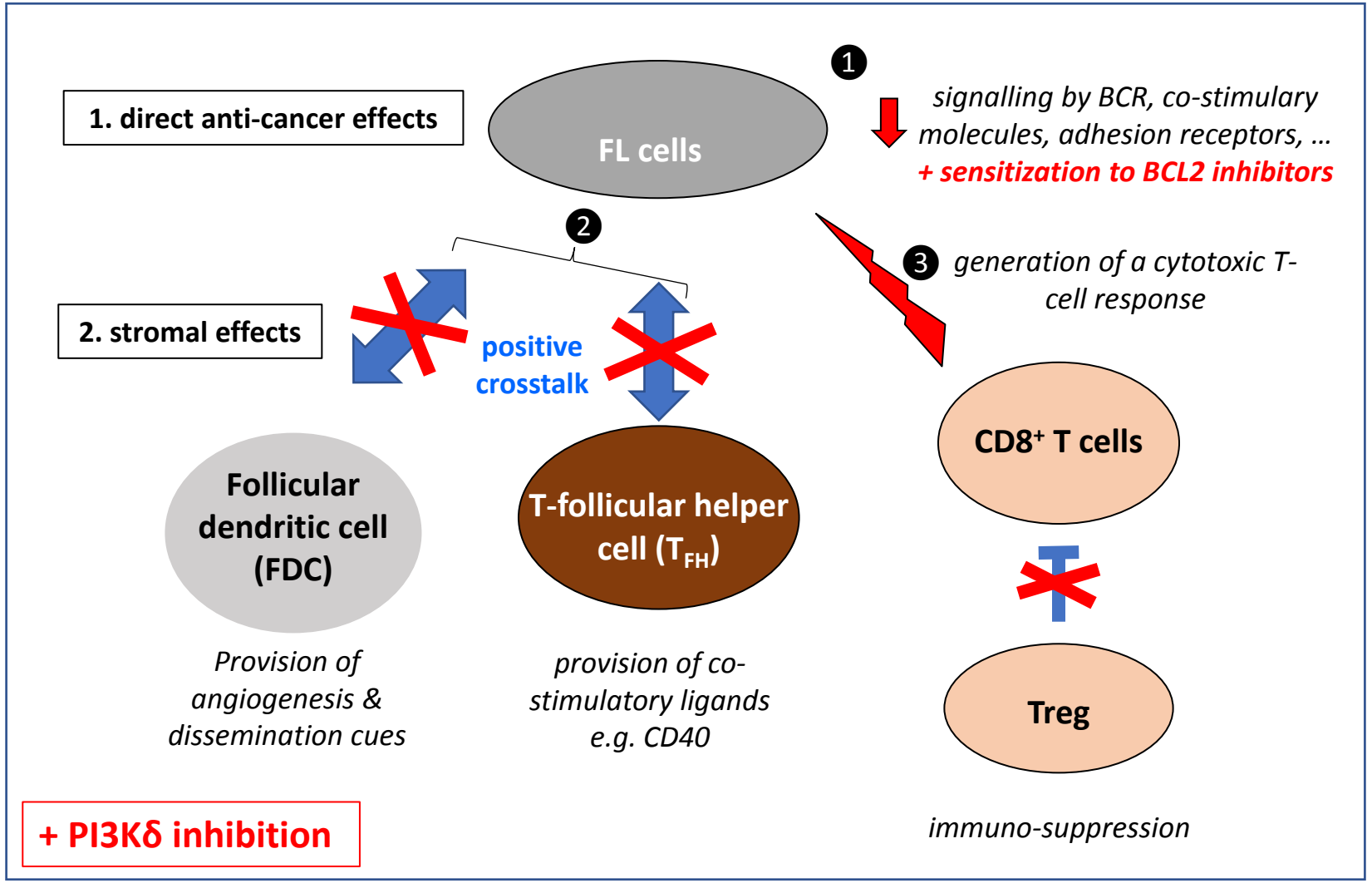


Figure 7: Anti-tumour mechanisms of PI3Kδ inhibition

c. in solid tumours