nature reviews drug discovery

https://doi.org/10.1038/s41573-026-01388-5

Perspective

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A renaissance in targeting the PI3K/AKT/mTOR pathway

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Abstract

The PI3K/AKT/mTOR pathway represents an excellent drug target, given its proven druggability and roles in cancer, immune regulation and genetic disorders. However, although some agents targeting this pathway have gained approval, drug development has faced challenges due to tolerability issues and resistance mechanisms, amongst other obstacles. New pharmacological approaches and improved biological and clinical understanding are now renewing interest and confidence in therapeutically modulating PI3K/AKT/mTOR signalling. This Perspective covers the challenges, lessons learned and new efforts in targeting this pathway.

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Introduction

Despite extensive PI3K/AKT/mTOR inhibitor development efforts, only a limited number of compounds have been approved by the US Food and Drug Administration (FDA) (Table 1). The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1)-targeting rapamycin and its analogues are the most well-established inhibitors of this pathway, the first of which was approved by the FDA in 1999 as an immunosuppressive agent. More recent developments have led to the discovery of inhibitors of upstream kinases, and in the last 11 years eight inhibitors — idelalisib, umbralisib, duvelisib and copanlisib for haematological malignancies, leniolisib for immunodeficiency and alpelisib, inavolisib and capivasertib for breast cancer — have gained FDA approval. The clinical progression of these and other molecules targeting the PI3K/AKT/mTOR pathway has been hindered by on-target toxicities, given that the pathway not only drives disease but is also involved in the maintenance of normal cellular functions. Progress has also been hampered by regulatory and clinical issues, including competition from agents that target alternative pathways within the same disease setting, and a regulatory approach to assess all compounds as a class rather than based on individual characteristics.

Approaches to target this pathway beyond the classical ATP-competitive inhibitors are now emerging, including allosteric and mutant-selective inhibitors, degraders, RAS–PI3Kα breakers and new mTOR inhibitors (Table 2). PI3K/AKT/mTOR pathway activation could also be of therapeutic benefit, for example in tissue protection, regeneration and immune activation, and pharmacological PI3K pathway activators have been reported.

Three classes of PI3K isoforms have been defined. This Perspective will focus on class I PI3Ks and their downstream signalling, given their extensive roles in disease and the substantial drug development efforts to target these enzymes. Class I PI3Ks produce the second messenger lipid PI(3,4,5)P_3 (PIP_3), which recruits and activates lipid-binding effectors such as the protein kinase AKT to regulate numerous cellular functions including growth, proliferation, differentiation, migration and metabolism[1] (Fig. 1 and Box 1).

Class I PI3Ks (PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ) are activated by receptor tyrosine kinases (RTKs), G-protein-coupled receptors (GPCRs) and small GTPases including RAS (Fig. 1). These heterodimeric PI3Ks consist of a catalytic subunit (p110α, p110β or p110δ, encoded by *PIK3CA*, *PIK3CB* and *PIK3CD*, respectively) bound to a p85 regulatory subunit (p85α, p85β or p55γ, encoded by *PIK3R1*, *PIK3R2* and *PIK3R3*, respectively). The p110γ catalytic subunit is complexed to the p84 or p101 regulatory subunits which have no homology to p85 proteins.

p110α and p110β are ubiquitously expressed; in contrast, p110γ and p110δ are highly enriched in leukocytes. Interestingly, p110δ can also become highly expressed in some solid tumour types, such as melanoma and breast cancer[2,3]. The class IA PI3K isoforms are recruited and activated by diverse stimuli, with p110α most sensitive to activation by RAS and RTKs, tuning it for insulin signalling. p110β integrates RTK, GPCR and small GTPase signals. p110δ is activated by the T cell antigen receptor (TCR), B cell antigen receptor (BCR) and chemokines, amongst others, and p110γ is mainly activated by GPCRs (Fig. 1).

This pathway is frequently hyperactivated in cancer, overgrowth, metabolic and immunodeficiency syndromes. This is due to activating mutations (in PI3K subunits, AKT isoforms (most commonly E17K in *AKT1*) or *MTOR*), loss-of-function mutations in the regulatory phosphatases (such as *PTEN*) or via non-genetic mechanisms. The class I PI3K/AKT/mTOR pathway is one of the most frequently aberrantly activated pathways in cancer, with activating *PIK3CA* mutations present in approximately 8% of solid tumours and *PTEN* inactivation in more than 30% of cancers[4–7]. PI3K/AKT/mTOR activation is also a common mechanism of cellular resistance to a wide range of anticancer agents[8,9].

Amongst the class I PI3Ks, *PIK3CA* and *PIK3R1* frequently exhibit activating mutations in cancer, with a low frequency of *PIK3CD*-activating mutations in some haematological malignancies. Oncogenic *PIK3CA* mutations are most frequently found in the p110α helical domain (E542K and E545K; weaking the inhibitory interactions with p85) and in the kinase domain (H1047R; increasing membrane binding), collectively mimicking steps in the p110α natural activation process[10]. Homologous mutations in *PIK3CD* (E522K, E525K, E1021K) also lead to increased kinase activity[11,12]. Activating *PIK3CA* mutations mosaically arising early in development lead to *PIK3CA*-related overgrowth spectrum (PROS), a rare non-cancerous overgrowth condition often associated with vascular malformations[13–15]. Germline mutations in *PIK3CD* or *PIK3R1* underlie activated PI3Kδ syndrome (APDS)[16], a primary immune deficiency which predisposes to recurrent infections, bronchiectasis and leukaemia.

Early mouse gene targeting studies showed that deleting a class I PI3K gene does not always have same the biological impact as gene targeting strategies that inactivate the PI3K protein, revealing that PI3K proteins exhibit scaffolding functions in addition to their kinase activity[17–20]. For example, deletion of *Pik3cb* is embryonically lethal at embryonic day 3.5 but kinase-dead mice are only partially embryonically lethal later in development and some mice survive to adulthood with only fertility phenotypes[21–24]. In addition, *Pik3cg* knockout but not kinase-dead mice are sensitized to mechanical stress-induced cardiac damage via a p110γ–PDE3B multimolecular complex that promotes phosphodiesterase activity and cAMP hydrolysis in a kinase-independent manner[20,25].

The class II and class III PI3Ks are less well characterized and indirectly control cell signalling via regulation of membrane trafficking and autophagy[26,27]. Class II PI3Ks (PI3K-C2α, PI3K-C2β and PI3K-C2γ) produce PI(3)P and PI(3,4)P_2, whereas the class III PI3K (VPS34) generates PI(3)P. These family members also have kinase-independent scaffolding functions[17–19]. Although these enzymes are rarely mutated in human disease, emerging evidence suggests that their dysregulation can contribute to sporadic and genetic diseases[28].

ATP-competitive inhibitors of class II PI3Ks (refs. 29,30) and VPS34 (refs. 31–34) have been generated. However, these compounds have not been clinically developed and will not be discussed here. Preclinical studies indicate therapeutic potential for class II PI3K inhibition in thrombosis (PI3K-C2α)[35] and ischaemic stroke (PI3K-C2β)[36]. VPS34 inhibitors were developed to modulate autophagy in oncology, immunity and degenerative diseases but, as far as we are aware, are no longer under active development, in part related to on-target toxicity observed in preclinical studies[34,37].

Here we review the currently FDA-approved class I PI3K/AKT/mTOR pathway inhibitors and the biomarkers used in inhibitor development (Box 2). We reflect on the challenges and lessons learned during the development of compounds targeting this pathway and how these can be applied to widen the therapeutic window. Finally, we discuss the new classes of modulators that are emerging and anticipated to overcome some of the key challenges. These include mutant-selective inhibitors, covalent inhibitors, RAS–PI3Kα breakers, degraders, dual and bifunctional inhibitors as well as the development of small molecule PI3K/AKT/mTOR activators.

Table 1 | Summary of FDA-approved PI3K/AKT/mTOR inhibitors to date (January 2026)

Drug (company)	Disease indication	Monotherapy or combination	Comments	Refs.
PI3Kα inhibitors				
Alpelisib/NVP-BYL719/Piqray (Novartis)	*PIK3CA*-mutated, HR$^+$HER2$^-$ advanced breast cancer	Combination with fulvestrant	Second line	300,301
Inavolisib/GDC-0077/RG-6114/Itovebi (Roche)		Combination with palbociclib and fulvestrant	First line in patients who have progressed on or within 1 year of completing adjuvant endocrine therapy	302–306
PI3Kδ or dual PI3Kγ/δ inhibitors				
Idelalisib/CAL-101/Zydelig (Gilead)	CLL FL SLL	CLL: combination with anti-CD20 antibody rituximab FL: monotherapy SLL: monotherapy	Withdrawn for FL and SLL	307
Duvelisib/IPI-145/Copiktra (Secura Bio)	CLL FL SLL	Monotherapy	Withdrawn from the market for FL and SLL	308
Umbralisib/TGR-1202/Ukoniq (TG Therapeutics)	FL Marginal zone lymphoma	Monotherapy approval; combination with anti-CD20 antibody ublituximab was in development for CLL before discontinuation	Withdrawn from the market	309
Leniolisib/CDZ173/Joenja (Pharming)	APDS	Monotherapy	FDA and MHRA, but not EMA approved	310
Linperlisib/YY-2039 (Shanghai Yingli Pharmaceutical)	FL	Monotherapy	Approved in China, ongoing clinical trials in multiple countries	174,311
Pan-PI3K inhibitor				
Copanlisib/BAY 80-6946/Aliqopa (Bayer)	FL	Monotherapy	Withdrawn from the market	312
AKT inhibitor				
Capivasertib/AZD5363/Truqap (AstraZeneca)	*PIK3CA/AKT1/PTEN*-mutated, HR$^+$/HER2$^-$ advanced breast cancer	Combination with fulvestrant	Ongoing exploration in other solid tumour contexts including prostate cancer	89
mTORC1 inhibitors				
Sirolimus (rapamycin) (Pfizer)	Transplantation: immunosuppression Cancer	Combination therapy for transplantation Monotherapy for malignant perivascular epithelioid cell tumour	Allosteric inhibitor	64
Everolimus (Novartis)	Transplantation: immunosuppression Cancer: renal carcinoma, neuroendocrine tumours, breast cancer Tuberous sclerosis	Breast cancer with exemestane Neuroendocrine tumours as monotherapy Renal cell carcinoma with lenvatinib Tuberous sclerosis monotherapy or combination	Allosteric inhibitor	64
Temsirolimus (Pfizer)	Cancer: renal carcinoma	Monotherapy	Allosteric inhibitor	64

APDS, activated PI3Kδ syndrome; CLL, chronic lymphocytic leukaemia; FDA, US Food and Drug Administration; FL, follicular lymphoma; HR$^+$, hormone receptor-positive; MHRA, Medicines and Healthcare products Regulatory Agency; mTORC1, mechanistic target of rapamycin (mTOR) complex 1; SLL, small lymphocytic lymphoma.

FDA-approved PI3K/AKT/mTOR pathway inhibitors

PI3K pathway drug development has mainly focused on reversible ATP-competitive kinase inhibitors[5,6,38]. These efforts led to approval of inhibitors for rare diseases caused by PI3K pathway mutations and cancer (Table 1 and Fig. 2). Of the class I PI3Ks, PI3Kα and PI3Kδ have attracted most drug development and clinical attention, resulting in FDA-approved inhibitors of these PI3Ks. Numerous PI3Kβ and PI3Kγ inhibitors have also been developed and tested clinically in cancer (PI3Kβ (ref. 39) and PI3Kγ (refs. 40,41)) or as an anti-thrombotic (PI3Kβ (refs. 42–44)) but have not led to regulatory approval. Interestingly, PI3Kβ inhibitors are remarkably well tolerated in humans (at doses for which target engagement was observed in platelet-rich plasma, in tumours or via impacts on coagulation parameters[39,43,44]) compared with other isoform-specific PI3K inhibitors, perhaps because PI3Kβ may play a less critical role in adult homeostasis, as also suggested by mouse studies[21–24].

PI3Kα/AKT inhibitors

Advanced breast cancer is the only solid tumour indication for which PI3K/AKT inhibitors have been FDA-approved. Approximately 40% of hormone receptor-positive (HR$^+$) breast cancers contain activating *PIK3CA* mutations, with PI3Kα playing a key role in both tumour development and hormone sensitivity. This has been exploited by combining PI3Kα/AKT inhibitors with the oestrogen receptor (ER) degrader fulvestrant[45–47] (Fig. 3). A key mechanism of action likely involves anti-proliferative[48]/anti-metabolic effects of PI3K/AKT inhibitors on breast cancer cells, inducing compensatory ER expression via transcriptional and epigenetic mechanisms (release of FOXO-driven inhibition of ER transcription[46] and induction of an open chromatin state of the ER gene locus by the KMT2D histone methyltransferase[47]).

FDA-approved inhibitors in HR$^+$ advanced breast cancer are the PI3Kα inhibitors alpelisib/BYL719/Piqray (Novartis) and

Table 2 | PI3K/AKT/mTOR inhibitors in clinical development (January 2026)

Drug (company)	Disease indications investigated in trials	Trial status/ID	Refs.
PI3Kα inhibitors (not mutant-selective)			
TOS-358 (Totus Medicines)	Various solid tumours	Phase I recruiting (NCT05683418)	313
Risovalisib mesylate/CYH33 (Haihe Biopharma)	PROS and *PIK3CA*-related vascular malformations	Phase I/II recruiting (NCT06975618)	314
Serabelisib/FTH-001/INK-1117/MLN1117/TAK-117 in combination with sapanisertib/FTH-003/INK128/MLN0128/TAK-228 (Faeth Therapeutics)	Advanced or recurrent endometrial cancer	Phase II recruiting (NCT06463028) — with sapanisertib and paclitaxel with an insulin-suppressing diet	156,157
JS105 (Junshi Biosciences)	Solid tumours	Phase Ib/II recruiting (NCT06208410) Phase III recruiting (NCT07207070) — with dalpiciclib and fulvestrant	315
HS-10352 (Jiangsu Hansoh)	Breast cancer	Phase Ib/II not yet recruiting (NCT06788197) — SHR-A1811 and fulvestrant ± HS-10352	316
PI3Kα inhibitors (mutant-selective)			
RLY-2608/zovegalisib (Relay Therapeutics)	Solid tumours, advanced *PIK3CA*-mutant HR$^+$/HER2$^-$ breast cancer, PROS	Phase II recruiting (NCT05216432) — solid tumours and breast cancer with fulvestrant Phase III recruiting (NCT06982521) — *PIK3CA*-mutant HR$^+$/HER2$^-$ breast cancer with fulvestrant compared with capivasertib + fulvestrant Phase II recruiting (NCT06789913) — PROS	201
STX-478/LY4064809/Tersolisib (Scorpion Therapeutics)	Solid tumours	Phase I/II recruiting (NCT05768139) Phase III recruiting (NCT07174336) — breast cancer in combination with other treatments Phase I trial not yet recruiting (NCT07339735) — healthy participants	202
ETX-636 (Ensem Therapeutics)[a]	Metastatic breast cancer	Phase I/II recruiting (NCT06993844) — monotherapy or with fulvestrant	317
OKI-219 (OnKure)[a]	Metastatic cancer/metastatic breast cancer	Phase I recruiting (NCT06239467) — monotherapy or with fulvestrant or trastuzumab	318
PI3Kδ inhibitors			
Roginolisib/IOA-244 (iOnctura)	Solid tumours, haemato-oncology	Phase I active not recruiting (NCT04328844) — metastatic cancers Phase II recruiting (NCT06717126) — uveal melanoma Phase I/II recruiting (NCT06879717) — NSCLC ± docetaxel Phase I/II recruiting (NCT06887803) — myelofibrosis Phase I/II recruiting (NCT06644183 — chronic lymphocytic leukaemia (CLL) with venetoclax and rituximab Phase I recruiting (NCT07203391) — uveal melanoma with tebentafusp Phase I/II not yet recruiting (NCT07018752) — peripheral T cell lymphomas	319,320
Amdizalisib/HMPL-689 (HUTCHMED)	Haemato-oncology	Phase I terminated (NCT03786926)	321
BGB-10188 (BeOne Medicines, formerly BeiGene)[a]	Solid tumours and B cell malignancies	Phase I/II completed (NCT04282018) — with tislelizumab	322
Dual PI3Kγ/δ inhibitor			
Tenalisib/RP6530 (Rhizen)	TNBC	Phase II recruiting (NCT06189209) —TNBC	323
Dual PI3Kβ/δ inhibitor			
CVL-237/KA2237 (Convalife)	APDS, solid tumours	Phase II/III not yet recruiting (NCT06293716) — APDS Phase II not yet recruiting (NCT06183736) — solid tumours with *PTEN* deficiency	324,325
TBO-309 (AZD-6482) (ThromBio)	Acute ischaemic stroke	Phase II recruiting (NCT06813651) Phase II recruiting (NCT05363397)	44

Table 2 (continued) | PI3K/AKT/mTOR inhibitors in clinical development (January 2026)

Drug (company)	Disease indications investigated in trials	Trial status/ID	Refs.
PI3Kγ inhibitor			
Eganelisib/IPI-549 (Stelexis BioSciences)	Acute myeloid leukaemia	Phase I recruiting (NCT06533761)	326
RAS–PI3Kα breakers			
BBO-10203 (BridgeBio Oncology Therapeutics)	Solid tumours	Phase I recruiting (NCT06625775)	264
VVD compound (Vividion Therapeutics)[a]	Solid tumours	IND enabling stage	265
AKT inhibitors			
Afuresertib (Laekna)	Breast cancer	Phase III recruiting (NCT04851613)	327
Engasertib/VAD044 (Vaderis/Almac)	Vascular malformations — hereditary haemorrhagic telangiectasia	Phase Ib active, not recruiting (NCT05406362)	182
Miransertib/ARQ 092/MK-7075 (ArQule)	Vascular malformations, Proteus syndrome	Phase II active, not recruiting (NCT04980872) Phase II recruiting (NCT04316546)	178,179
ATP-competitive mTORC1/2 inhibitor			
Sapanisertib/FTH-003 (Faeth Therapeutics)	Bladder cancer, endometrial cancer, liver cancer	Phase II active, not recruiting (NCT03047213) — bladder cancer with *TSC1* and/or *TSC2* mutations Phase II recruiting (NCT06463028) — endometrial cancer with serabelisib Phase I/II recruiting (NCT06811116) — liver cancer with chemotherapy Phase I/Ib active, not recruiting (NCT04250545) — NSCLC with Telaglenastat Phase I active, not recruiting (NCT02503722) — NSCLC with osimertinib Phase I active, not recruiting (NCT02142803) — glioblastoma or solid tumours with bevacizumab Phase II active, not recruiting (NCT06390865) — cancers with *TSC1* or *TSC2* mutations Phase II active, not recruiting (NCT06385496) — cancers with *MTOR* mutations	157
Bisteric mTORC1 inhibitor			
RMC-5552 (Revolution Medicines)	Glioblastoma	Phase I/Ib recruiting (NCT05557292, NCT05557292)	248
Pan-class I PI3K/mTOR inhibitors			
Gedatolisib (Celcuity)	Solid tumours	Phase I recruiting (NCT03065062) — squamous cell lung, pancreatic, head and neck, and other solid tumours with palbociclib Phase I/II active, not recruiting (NCT03911973) — triple negative or BRCA1/2$^+$, HER2$^-$ breast cancers with talazoparib Phase I/II recruiting (NCT06190899) — castration-resistant prostate cancer with darolutamide Phase III active, not recruiting (NCT05501886) — HR$^+$/HER2$^-$ breast cancer with fulvestrant ± palbociclib Phase III recruiting (NCT06757634) — first-line treatment for HR$^+$HER2$^-$ breast cancer with fulvestrant + CDK4/6 inhibitor Phase II recruiting (NCT03675893) — endometrial or serous ovarian cancer with abemaciclib + letrozole ± metformin, zotatifin or gedatolisib	140,249
Bimiralisib (TORQUR)	Actinic keratosis	Phase II completed (NCT06319794)	132
Paxalisib/GDC-0084 (Kazia/Sovargen)	Glioblastoma multiforme, other gliomas, primary central nervous system lymphoma, metastatic solid tumour	Phase II recruiting (NCT04906096) — primary central nervous system lymphoma Phase I/II recruiting (NCT06208657) — paediatric cancer Phase II recruiting (NCT05009992) — diffuse midline glioma, ONC201 ± panobinostat or paxalisib Phase II/III recruiting (NCT03970447) — glioblastoma Phase I/II not yet recruiting (NCT07287202) — focal cortical dysplasia type II, tuberous sclerosis complex or hemimegalencephaly	328

Only compounds under active clinical development are listed. APDS, activated PI3Kδ syndrome; HR$^+$, hormone receptor-positive; mTORC1, mechanistic target of rapamycin (mTOR) complex 1; NSCLC, non-small cell lung cancer; PROS, *PIK3CA*-related overgrowth spectrum; TNBC, triple-negative breast cancer. [a]Structure and data not disclosed.

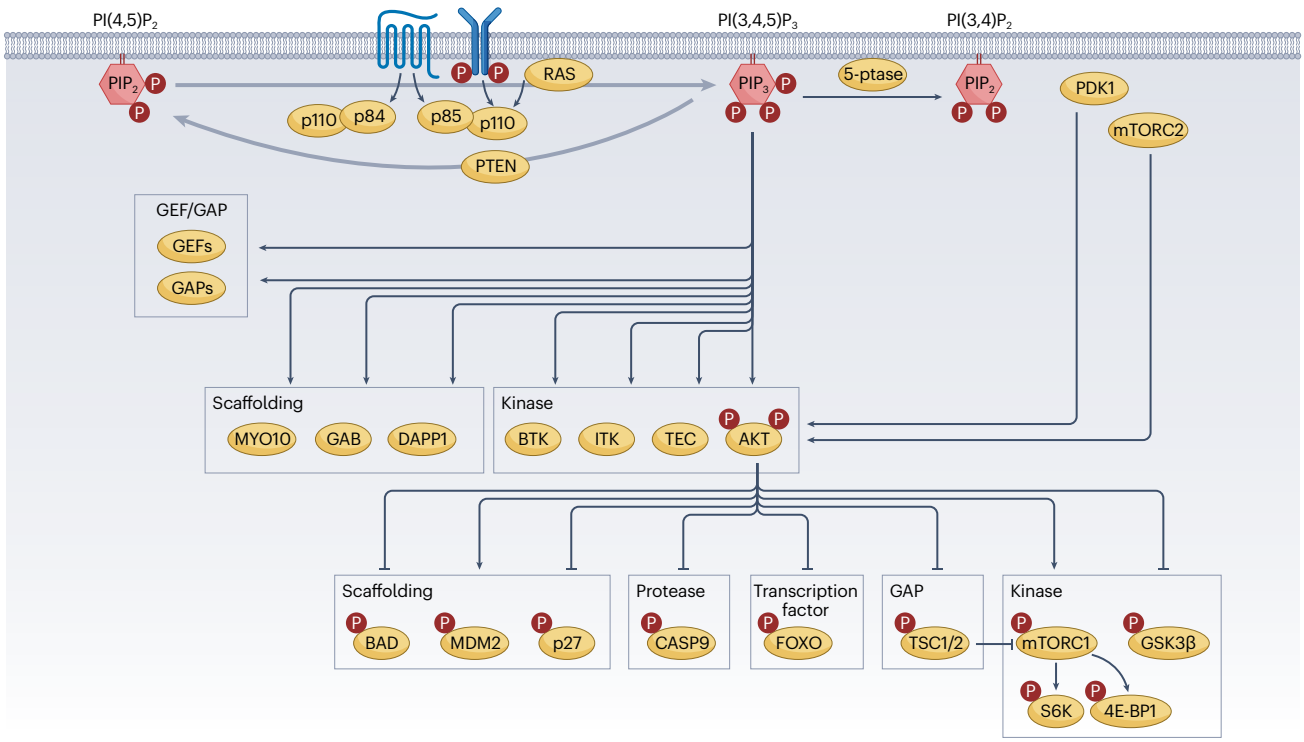


Fig. 1 | The class I PI3K signalling pathway. Class I PI3Ks are activated by receptor tyrosine kinases (RTKs), G-protein-coupled receptors (GPCRs) and small GTPases (including RAS, RAC, CDC42 and RAB5), leading to phosphorylation of $PI(4,5)P_2$ at the plasma membrane, producing $PI(3,4,5)P_3$ (PIP_3). PIP_3 and its derivative $PI(3,4)P_2$, generated by 5-phosphatases (5-ptase), recruit pleckstrin homology (PH) domain-containing effectors, such as GEFs/GTPase-activating proteins (GAPs), scaffolding proteins and protein kinases such as the Ser/Thr kinase AKT, which is then phosphorylated by phosphoinositide-dependent kinase 1 (PDK1) and mechanistic target of rapamycin (mTOR) complex 2 (mTORC2) resulting in its activation. Activated AKT is a critical effector of the class I PI3K pathway and phosphorylates numerous kinases, GAPs, transcription factors, proteases and scaffolding proteins to regulate cellular processes including proliferation, cell survival, metabolism, DNA repair and motility. AKT also regulates cell growth and translation via phosphorylation and inhibition of the mTORC1 inhibitors PRAS40 and TSC1/2. PTEN is a major negative regulator of the pathway, dephosphorylating PIP_3 and $PI(3,4)P_2$ at the 3-position. 4E-BP1, 4E-binding protein-1; S6K, ribosomal protein S6 kinase.

inavolisib/GDC-0077/Itovebi (Roche). Alpelisib is approved by the FDA for second-line treatment in combination with fulvestrant based on the SOLAR-1 trial[45] and is also effective in patients who have received first-line CDK4/6 inhibitors plus endocrine therapy based on the BYLieve trial[49]. Inavolisib is FDA-approved as first-line therapy in combination with palbociclib (CDK4/6 inhibitor) and fulvestrant in patients who progressed during or within 1 year of completing adjuvant endocrine therapy, based on the INAVO120 trial[50]. This inavolisib triplet therapy improves overall survival in HR^+ advanced breast cancer[51] from 27 to 34 months, which is the first time this milestone has been reached for a PI3K pathway inhibitor in metastatic breast cancer[52].

The ongoing INAVO121 trial (NCT05646862) is a randomized phase III study comparing inavolisib with fulvestrant versus alpelisib and fulvestrant in second-line advanced breast cancer[53]. Additional trials of inavolisib in progress in breast cancer are INAVO123 (NCT06790693, phase III randomized controlled trial) testing palbociclib and letrozole (aromatase inhibitor) ± inavolisib in patients with endocrine-sensitive *PIK3CA*-mutated HR^+ advanced breast cancer; a phase I trial (NCT05894239) testing inavolisib with subcutaneous trastuzumab and pertuzumab (anti-HER2 antibodies, PHESGO) in *PIK3CA*-mutated HER2-amplified advanced breast cancer; and GeparPiPPa (NCT05306041, phase II randomized controlled trial) testing endocrine therapy with trastuzumab and pertuzumab ± inavolisib in early breast cancer.

Alpelisib is also FDA-approved as monotherapy for PROS[13,14], with confirmed objective responses in the EPIK-P2 prospective phase II study of 16.7% and 23.2% in adult and paediatric patients, respectively[54].

Multiple AKT inhibitors have been developed, either ATP-competitive or pleckstrin homology (PH) domain-dependent non-ATP-competitive (reviewed in refs. 55,56). Only the ATP-competitive pan-AKT inhibitor capivasertib/AZD5363/Truqap (AstraZeneca) has received clinical approval, namely in combination with fulvestrant for hormone-resistant HR^+/$HER2^-$ breast cancer with mutations in *PIK3CA, AKT1* or *PTEN*[57]. Although capivasertib has some adverse effects (for example, rash and diarrhoea), it is currently more commonly used in clinical practice than alpelisib, amongst other reasons due to the lower impact of hyperglycaemia in the 4 days on/3 days off dosing scheme used for capivasertib. Capivasertib continues to be clinically tested in endocrine-dependent breast and prostate cancer. The ongoing CAPItello-292 trial (NCT04862663) is now investigating capivasertib in the first-line setting in combination with CDK4/6 inhibitors and fulvestrant, in a patient population similar to that of the INAVO120 trial. The CAPItello-281 trial (NCT04493853) in metastatic hormone-sensitive *PTEN* deficient prostate cancer testing capivasertib

in combination with abiraterone–prednisone and androgen deprivation therapy showed a 7.5 month statistically significant improvement in radiographic progression-free survival[58].

PI3Kδ inhibitors

Inhibitors targeting PI3Kδ (idelalisib/CAL-101/Zydelig (approved for chronic lymphocytic leukaemia (CLL)) and umbralisib/TGR-1202/Ukoniq (withdrawn)), PI3Kγ/δ (duvelisib/IPI-145/Copiktra (approved for CLL)) or pan-class I PI3K (copanlisib/BAY 80-6946/Aliqopa (withdrawn)) have been FDA-approved for relapsed B cell malignancies (Fig. 3). These include CLL, for which both idelalisib and duvelisib have full approval and continue to be available, and low-grade lymphomas including follicular lymphoma (FL), small lymphocytic lymphoma (SLL) and marginal zone lymphoma for which all inhibitors have been withdrawn. In all cases, although some compounds inhibit multiple isoforms, PI3Kδ was the target isoform. Although effective, the clinical use of these compounds is limited due to toxicities and the availability of better-tolerated alternative targeted therapies, such that all four drugs that had accelerated approvals in low-grade lymphomas have been withdrawn from the market for that indication, with umbralisib and copanlisib no longer available[59,60].

Although *PIK3CD* is not mutated in CLL and FL, these malignancies have a specific dependency on chronic cell-intrinsic activation of the BCR and on microenvironmental stimuli, both of which to a large extent depend on PI3Kδ, together with BTK and SYK[6,61,62]. PI3Kδ inhibition also suppresses leukaemia-supporting cells in the microenvironment (reviewed elsewhere[6]). This triple dependency may explain the superior clinical impact of PI3Kδ inhibition in CLL and FL, compared with other haematological malignancies which also express PI3Kδ.

The PI3Kδ inhibitor leniolisib (CDZ173/Joenja (Novartis/Pharming)) is FDA-approved as monotherapy for APDS[16]. In this condition, leniolisib is well tolerated and leads to normalization of immune defects and improvements in health-related quality of life, positioning this drug as a long-term therapeutic option for the treatment of APDS[63].

mTOR inhibitors

The mTORC1 inhibitor rapamycin (also known as sirolimus) is a natural product which, along with its derivatives called rapalogs, are FDA-approved for immunosuppression, oncology (Fig. 3) and rare diseases[64]. The first genetic disorder associated with the PI3K/AKT/mTOR pathway was inactivating mutations in the tuberous sclerosis complex proteins *TSC1* or *TSC2* which inhibit mTORC1, with rapalogs now FDA-approved to treat this condition[65].

Lessons learned and ongoing challenges in targeting PI3K/AKT/mTOR

On-target toxicities limit drug tolerability and treatment continuation

Inhibition of the PI3K/mTOR pathway is linked to metabolic and immune on-target adverse effects. The first is hyperglycaemia, resulting from the key role of PI3Kα and AKT in insulin signalling[66]. This is reflective of murine genetic models whereby *Pik3ca*

Box 1 | The PI3K/AKT/mTOR pathway: signalling molecules, mechanisms and contexts

Class IA PI3Ks are heterodimers consisting of a p110 catalytic subunit (p110α, p110β or 110δ) and a p85 regulatory subunit. The p85 regulatory subunits are critical for the stability of the catalytic subunits, maintaining them in an inactive conformation and also promoting activation in response to upstream stimulation (reviewed elsewhere[329]). Although p110α and p110β bind p85α and p85β with similar stoichiometry, p110δ preferentially associates with p85α (ref. 330). It has recently been shown that the activity and dynamics of p110α are not differentially modulated by the different p85 regulatory subunits[331]. The class IB isoform p110γ is regulated by p84 or p101.

Activated class I PI3Ks phosphorylate the $PI(4,5)P_2$ membrane inositol lipid to generate $PI(3,4,5)P_3$ (PIP_3) which can be converted to $PI(3,4)P_2$ by 5-phosphatases. PTEN is a major negative regulator of the pathway, dephosphorylating PIP_3 and $PI(3,4)P_2$ at the 3-position. PIP_3/$PI(3,4)P_2$ activate protein kinases such as the Ser/Thr kinases AKT1, AKT2 and AKT3 and several other lipid-binding domain-containing effectors, including tyrosine kinases (for example, BTK, ITK, TEC), adaptor proteins (for example, DAPP1, GAB1, SKAP1, KINDLIN-3) and regulators of proteins of the small GTPase superfamily, including GEFs and GTPase-activating proteins (GAPs) of the RAS, RHO, RAB and ARF family[332,333] (Fig. 1).

AKT1, AKT2 and AKT3 are key PI3K effectors recruited to the plasma membrane by their PIP_3-binding pleckstrin homology (PH) domains, where they are activated via phosphorylation on Thr308 (by phosphoinositide-dependent kinase 1 (PDK1)) and Ser473 (by the mechanistic target of rapamycin (mTOR) complex 2 (mTORC2)). Assessment of these phosphorylations in AKT (referred to as pAKT) is the most commonly used biological read-out for PI3K pathway activity. AKT kinases phosphorylate and modulate the activity of more than 100 reported substrates, such as mTORC1, BAD, MDM2, FOXO, GSK3β, caspase 9 and p27, involved in a broad range of biological activities and most often acting by inactivating negative regulators[1,334] (Fig. 1). Here we will focus on PI3K/AKT/mTORC1 signalling as these nodes have been the key focus of drug development efforts.

The Ser/Thr kinase mTOR exists in two distinct complexes with diverse functions[1,335] (Fig. 1). The mTORC1 complex[336] (characterized by the presence of RAPTOR and PRAS40) integrates signalling from a range of stimuli, including growth factors (which act on mTORC1 via PI3K/AKT), nutrients (such as amino acids) and cellular energy levels. The mTORC2 complex (characterized by the presence of RICTOR, PROTOR and mSIN1) plays an important role in activation of AKT, SGK1, PKCα and other kinases[335,337].

AKT promotes mTORC1 activation by inactivating its inhibitors: it phosphorylates PRAS40 to relieve its inhibitory effect on mTORC1 and phosphorylates the TSC1/2 complex to suppress its GAP activity for the RHEB small GTPase, thereby keeping RHEB activation in its active GTP-bound state to allosterically activate mTORC1 on the lysosome[1] (Fig. 1).

Key mTORC1 substrates are p70 ribosomal protein S6 kinase (S6K) and 4E-binding protein-1 (4E-BP1), which regulate ribosome biogenesis and translation initiation, respectively (Fig. 1). Other mTORC1 effectors inhibit autophagy or promote synthesis of proteins, lipids and nucleotides[335].

Box 2 | Biomarkers for PI3K/AKT/mTOR inhibitors

Extensive work has been reported on the development of predictive biomarkers for patient selection and target engagement in specific indications. Unfortunately, no biomarkers for resistance to PI3K/AKT/mTOR inhibitor treatment have been established.

Biomarkers for patient selection

For the US Food and Drug Administration (FDA)-approved PI3Kα and AKT inhibitors alpelisib, inavolisib and capivasertib, the biomarkers for patient selection are well established. Only patients with hormone-resistant $HR^+/HER2^-$ breast cancer with mutations in *PIK3CA*, *AKT1* or *PTEN* are eligible for these inhibitors[338]. The key role PI3Kβ plays in *PTEN*-mutant tumours has led to the suggestion that *PIK3CB* and *PTEN* mutations may be potentially useful biomarkers for PI3Kβ inhibitor patient selection in oncology settings[339] (NCT03218826). The absence of MYC expression has also been suggested as a biomarker for response to the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) inhibitor everolimus in $HR^+/HER2^-$ breast cancer[340]. To the best of our knowledge, biomarkers for patient selection have not been implemented for PI3Kδ inhibitors in leukaemia and lymphoma. However, a recent study proposed that the levels of a set of five phosphoproteins (pBcl-2(S70), pMEK1(S298), pSTAT1(Y701), p90 RSK(S280) and p38 MAPK(T180/Y182)) in peripheral blood mononuclear cells prior to treatment distinguish umbralisib responders from non-responders, and may also be applicable to other PI3Kδ inhibitors[341].

Pharmacodynamic biomarkers

Identifying reliable pharmacodynamic biomarkers for PI3K/AKT/mTOR inhibition has remained a challenge for the field. Analysis of the activation status of downstream PI3K pathway effectors such as pAKT, pPRAS40, pGSK3β, pS6RP and p4E-BP1 is an obvious approach to quantify target engagement following PI3K pathway inhibition. However, this has proven technically challenging due to difficulties in sampling solid tumours, with skin biopsies often not representative of the tumour, and inconsistent sample preparation in multi-site clinical studies preventing accurate quantification (reviewed elsewhere[342]). Measurement of insulin and glucose homeostasis has also been explored as a pharmacodynamic read-out of PI3Kα and AKT inhibition but has also not proved to be a reliable marker of drug response (reviewed elsewhere[342]).

In terms of PI3Kδ inhibition, a robust measure of target engagement in preclinical studies is depletion of the marginal zone B cells and regulatory T cells (T_{reg} cells) in the spleen, mirroring the phenotype of *Pik3cd* kinase-dead mice[72,343]. However, spleen sampling is not feasible in the clinical setting. Alternatives such as ex vivo basophil activation in peripheral blood samples (using BASOTEST, given the key role of PI3Kδ in FcεRI signalling[112,113]) and ex vivo stimulation of B cells have also been explored. Indeed, the dosing strategy used for leniolisib was based on pAKT levels in ex vivo-stimulated activated PI3Kδ syndrome (APDS) versus control B cells[344].

kinase-dead mice exhibit impaired glucose and insulin tolerance and hyperinsulinaemia[67], and *Akt2* knockout mice exhibit hyperglycaemia and glucose intolerance[68]. The second is disruption of immune homeostasis upon inhibition of PI3Kγ/δ (refs. 6,59,60,69) or mTOR[70], resulting in adverse immune effects, including opportunistic infections and autoimmune-like responses (Box 3). Similarly, *Pik3cg* deletion in mice disrupts T cell activation and neutrophil function[71], *Pik3cd* kinase-dead mice have impaired adaptive immune signalling/responses and disrupted immune tolerance[72], and *Mtor* hypomorphic mice exhibit defective adaptive immune development and signalling[73]. Mucositis/stomatitis has also been a major adverse effect caused by mTORC1 inhibition limiting inhibitor use, particularly in chronic conditions[74], but this can be mitigated with dexamethasone mouthwash[75].

Immune-related adverse effects have been mainly observed with PI3Kδ inhibitors which, by preferentially inhibiting immunosuppressive regulatory T cells (T_{reg} cells), can disrupt immune tolerance. The notion that colonic T_{reg} cells appear to be particularly sensitive to PI3Kδ inhibition[76,77] may help explain why colitis is a key side effect of PI3Kδ inhibitors and is observed in homozygous *Pik3cd* kinase-dead mice[72]. It is important to note that PI3Kδ inhibitor-associated autoimmune-like effects are transient and resolve upon drug withdrawal[78–80].

Overall, it has become clear that there is a need to preserve a baseline level of PI3K activity to support immune homeostasis, especially in the colon. Indeed, although clinical comparative data are lacking, genetic studies in mice suggest that immune-related toxicities may be increased by dual targeting of PI3Kδ and PI3Kγ[81]). Likewise, upon dual PI3Kα/δ inhibition (as is the case for taselisib/GDC-0032), it has been suggested that PI3Kα inhibition-induced epithelial barrier dysfunction may synergize with PI3Kδ inhibitor-induced T effector cell activation to promote colitis[82].

Inhibition of cancer cell-intrinsic PI3K/AKT/mTOR is not directly cytotoxic

A major rationale for developing PI3K pathway inhibitors has been to block cancer cell-intrinsic PI3K/AKT/mTOR activity. Early studies used LY294002 and wortmannin, often at high doses, to probe for PI3K involvement in biological processes, including to show in vitro cytotoxic activity on cancer cells[8]. These early findings led to the widely held view that PI3K pathway inhibitors would be cytotoxic for cancer cells, which was further enhanced by misinterpretation of growth inhibition data obtained using ATP-based metabolic cell measurements as bona fide cytotoxic activity.

Subsequent studies revealed that LY294002 and wortmannin have multiple non-PI3K/AKT/mTOR-dependent targets. Studies using more selective PI3K pathway inhibitors and accurate assessments of drug effects[83,84] have now established that PI3K, AKT and mTOR inhibitors are cytostatic and do not induce cell death in vitro[83–90]. This also applies to the pan-PI3K/mTOR inhibitors omipalisib/GSK2126458 and bimarilisib/PQR309 (refs. 83,85,91). However, gedatolisib/PF-05212384 (Celcuity) has shown cytotoxic activity for cancer cells in vitro, which is proposed to be due to more complete inhibition of multiple pathways compared with other inhibitors[92,93]. Furthermore, mTOR inhibitors have also been shown to be cytotoxic in some haematological malignancies, but these studies have generally used very high inhibitor concentrations[94]. Potent covalent p110α inhibitors also induce G1 cell cycle arrest rather than cytotoxicity[95]. A recent report presented evidence that

cancer cell lines treated with the mTORC1/2 inhibitor sapanisertib (also known as INK128/MLN0128/TAK-228) undergo a reversible cell cycle arrest with features of persistence and embryonic diapause, a reversible state of suspended development induced by unfavourable nutrient conditions[96]. This aligns with genetic evidence that inactivation of the *PIK3CA* paralogue AGE-1 in *Caenorhabditis elegans* induces a dormant developmental state, without inducing cell death[97].

Cells are hard-wired to maintain PI3K/AKT/mTOR activity

Cells possess a myriad of mechanisms to maintain PI3K/AKT/mTOR pathway activity, either through intrinsic mechanisms or newly

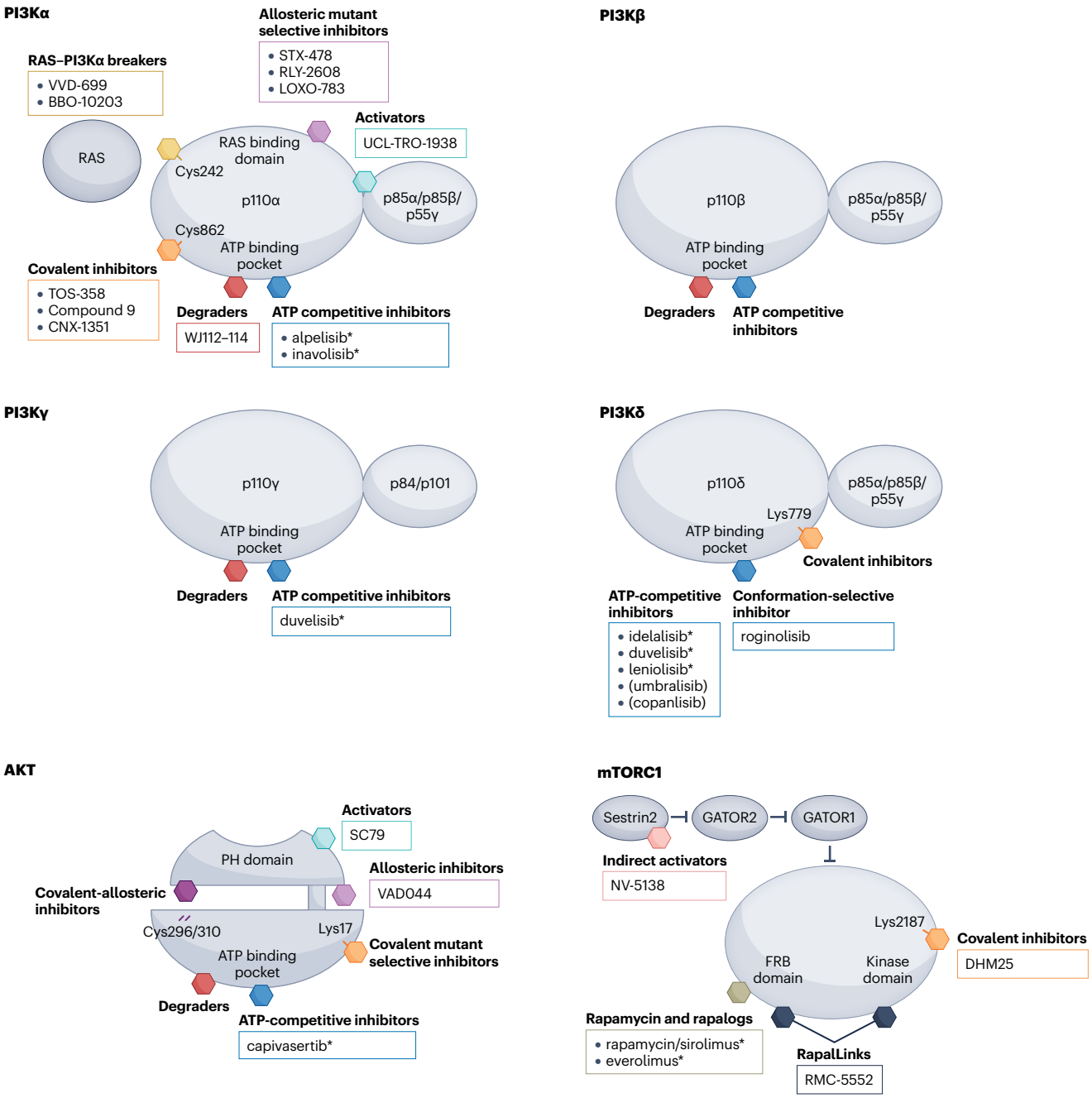
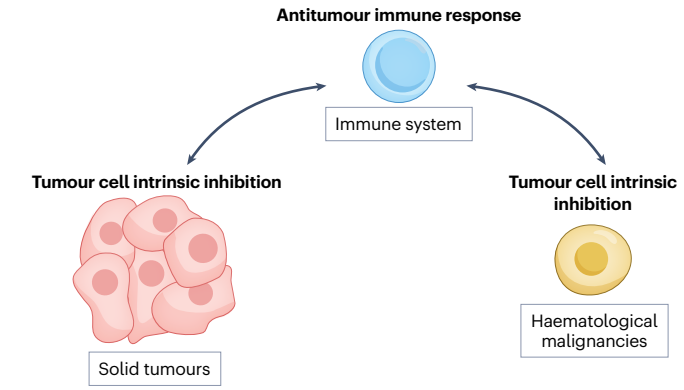


Fig. 2 | Mechanisms of action of small molecule class I PI3K pathway modulators. Established and emerging pharmacological methods to pharmacologically modulate class I PI3K isoforms, PI3Kα/PI3Kβ/PI3Kγ/PI3Kδ, and the pathway effectors AKT and mechanistic target of rapamycin (mTOR) complex 1 (mTORC1). Mechanisms of drug action, protein binding sites and key interacting residues are depicted, with the most clinically relevant compounds at present named. *Compounds currently US Food and Drug Administration (FDA)-approved. Compounds in parentheses are no longer clinically available. FRB, FKBP–rapamycin binding; PH, pleckstrin homology.



Inhibitor	Solid tumours	Immune system	Haematological malignancies
PI3Kα	⊕ (fulvestrant, palbociclib)	⊖	⊖
PI3Kβ	⊕	⊖	⊖
PI3Kγ	⊖	⊕	⊕
PI3Kδ	⊖	⊕	⊕ (rituximab)
AKT	⊕ (fulvestrant, palbociclib)	⊖	⊖
mTOR	⊕	⊕	⊖

Fig. 3 | Indications and mechanisms of action of PI3K/AKT/mTOR inhibitors in cancer. Solid tumours: PI3Kα and AKT inhibitors are US Food and Drug Administration (FDA)-approved for hormone receptor-positive (HR^+) advanced breast cancer and function to sensitize the tumour cells to oestrogen receptor (ER) degraders (fulvestrant) by inducing compensatory upregulation of ER expression. The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) inhibitor everolimus is also FDA-approved for HR^+ metastatic breast cancer and acts to inhibit tumour cell proliferation including via downregulation of ER expression. PI3Kβ inhibitors are being explored for solid tumours by targeting tumour cell-intrinsic PI3Kβ, particularly in the context of PTEN mutations where PI3Kβ plays a key role in stimulation of oncogenic signalling. Haematological malignancies: some B cell leukaemia and lymphoma cells are dependent on PI3Kδ signalling downstream of the B cell antigen receptor (BCR) and microenvironmental stimuli for maintenance in the bone marrow/lymph nodes. Upon PI3Kδ inhibition these cells move into the circulation, where they are susceptible to combination therapies such as anti-CD20 monoclonal antibodies (rituximab). PI3Kγ re-emerged in 2024 as an interesting target for acute leukaemia, particularly acute myeloid leukaemia, based on preclinical data, but the precise molecular mechanisms of the leukaemic cell PI3Kγ dependency remain unclear. Immunotherapy: PI3Kδ inhibition is under investigation for immunotherapy by preferentially inhibiting regulatory T cells (T_{reg} cells), leading to enhanced T effector cell antitumour immune responses. PI3Kγ may also be of interest as an immunotherapy agent by switching immunosuppressive tumour-associated macrophages to an immunostimulatory state via NF-κB activation and C/EBPβ inhibition, leading to a $CD8^+$ T cell antitumour response. It is also possible that PI3K/AKT/mTOR signalling in the tumour cells may alter their interaction with the microenvironment and thereby impact antitumour immune responses.

acquired genetic changes. This poses a huge challenge to the effective blockade of cancer cell-intrinsic PI3K pathway activity, indicating the need for combination therapy.

PI3K/AKT/mTOR signalling is tightly regulated by in-built feedback loops which keep signalling within the pathway balanced overall (reviewed elsewhere[98]). This is part of normal signalling homeostasis when cells are stimulated and the pathway becomes activated, with regulatory networks stepping in to dampen upstream signalling inputs. Conversely, when the pathway is inhibited in one node, this feedback inhibition is relieved, resulting in pathway reactivation. Of note, these feedback loops remain active in cancer cells and provide intrinsic drug resistance. For example, when mTORC1 is activated, it stimulates ribosomal protein S6 kinase (S6K) which then phosphorylates and inhibits the upstream IRS1 adaptor protein that mediates PI3K signalling downstream of the receptors for insulin and insulin-like growth factor. Thus, upon mTORC1 activation, feedback loops are initiated to dampen PI3K input into mTORC1. Reciprocally, mTOR inhibitors, other than dampening signalling downstream of mTORC1 as intended, hereby also take away these S6K-driven negative feedback loops, allowing cells to (hyper)activate growth factor/PI3K/AKT signalling, which can promote proliferation and resistance to mTORC1 inhibition[98].

Class I PI3K isoform-selective inhibition can also lead to compensatory (hyper)activation of non-targeted class I PI3K isoforms, amongst other mechanisms, via relief of inhibition on upstream RTKs[99–102]. Achieving a direct anticancer effect with isoform-selective PI3K inhibitor monotherapy is therefore challenging, with a combination of isoform-selective PI3K inhibitors shown to exert a greater antitumour response in preclinical models[99,100].

Other than activating a range of feedback loops, inhibition of mTORC1 can also activate autophagy, through loss of its inhibitory phosphorylation of the autophagy-activating kinase 1 (ULK1). Autophagy plays a complex role in cancer as it acts to both sensitize cells to therapy and confer resistance[103,104]. Therefore, autophagy activation by mTORC1 inhibition can induce resistance to other treatments.

Resistance and reactivation of the pathway can also be acquired at the genetic level by new compensating mutations in the target of the inhibitor or in downstream effectors (reviewed elsewhere[6]). A study on clinical resistance that developed in a patient with a *PIK3CA*-mutant breast cancer treated with alpelisib showed PTEN loss in different metastases by convergent evolution[105]. A cell-based study identified 63 putative alpelisib resistance genes, including activation of the PIM Ser/Thr kinases[106].

PI3Kδ inhibitor-induced antitumour immune responses likely do not require PI3Kδ inhibition at the maximum tolerated dose

An unexpected discovery has been that PI3Kδ inhibition exerts immunostimulatory activity, by preferentially dampening immunosuppressive T_{reg} cells over effector T cell subsets. This results in adaptive immune rebalancing towards a host anticancer T cell response, as first shown in mice[107] and validated in human CLL[108] and in a head and neck cancer window trial[77]. This finding uncovered the potential use of PI3Kδ inhibitors in cancer immunotherapy.

A key challenge is how to harness this therapeutic potential of PI3Kδ inhibition in light of its on-target adverse immune effects. A key consideration is that the immunomodulatory PI3Kδ inhibitor dose is likely to be lower than that aimed at blocking cancer cell-intrinsic PI3Kδ activity. This is illustrated by the clinical observations in patients with head and neck cancer, where the PI3Kδ inhibitor AMG319 (Amgen) was given at the maximum tolerated dose previously determined in patients with CLL aimed at blocking PI3Kδ in the leukaemic cells, in the context of heavy pretreatment and, most likely, immunosuppression[109]. In treatment-naive immunocompetent patients with head and neck cancer, severe immune-related side effects were observed within days of AMG319 administration, necessitating trial termination[77]. Treatment of immunocompetent tumour-bearing mice with an equivalent PI3Kδ inhibitor dose also led to immune-mediated adverse effects, which

were not observed upon intermittent dosing while maintaining an antitumour immune response[77,110].

Roginolisib/IOA-244 (iOnctura) has shown promising tolerability and monotherapy efficacy in patients with uveal, cutaneous melanoma and follicular non-Hodgkin lymphoma[111]. The roginolisib dose was selected to inhibit PI3Kδ by 90% based on basophil activation in peripheral blood (using BASOTEST, given the key role of PI3Kδ in FcεRI signalling[112,113]). At these doses, roginolisib did not induce the adverse immune effects that have plagued other PI3Kδ inhibitors and has been given long term to patients without prophylaxis, although we await the outcome of larger scale trials[111]. A key outstanding question is why other potent PI3Kδ inhibitors such as seletalisib/UCB5857 (UCB) and parsaclisib/INCB050465 (Incyte), despite dose reductions, still showed toxicities[114,115]. It is possible that the pharmacokinetic profile of roginolisib, with a low peak-to-trough ratio[111], its differentiated mechanism of p110δ inhibition providing increased selectivity for PI3Kδ over other PI3K isoforms (see below), and the lack of active metabolites (as was shown for idelalisib[116,117]) contribute to its capacity for chronic non-toxic dosing[118].

Interestingly, in rats and dogs, high doses of roginolisib do induce the on-target adverse effects previously associated with PI3Kδ inactivation, including gastrointestinal and skin toxicities[119]. It is therefore tempting to speculate that partial PI3Kδ inhibition may induce an effective host antitumour immune response without overt toxicities; seletalisib and parsaclisib did not achieve such effects because the tested doses of these highly potent compounds may still have been too high; and rebalancing immune responses is a biological parameter that cannot be achieved instantaneously, with the immunomodulatory dose of a PI3Kδ inhibitor not being the same as the maximum tolerated dose. In summary, partial rather than complete dampening of PI3Kδ activity may be required to achieve the desired biological outcome of immune rebalancing, with high doses of very potent inhibitors in fact being undesirable.

The setting of constitutive pathway activation by mutant PI3Kδ in APDS provided a well-defined target for dose-finding studies for the potent inhibitor leniolisib[120]. In a dose escalation study, the first dose that was found to reduce PI3K/AKT pathway activity in ex vivo-stimulated B cells from patients with APDS to within the normal range of unstimulated pAKT levels in healthy controls was used for follow-up in clinical trials[120,121]. Leniolisib as monotherapy is well tolerated in APDS and is emerging as a long-term therapeutic option for this condition[63]. At present, it is unknown whether the APDS leniolisib dose impacts the T_{reg} cells in patients with APDS or healthy subjects and therefore whether the favourable tolerability is due to the dose or is a feature of this molecule. It would also be interesting to test other potent PI3Kδ inhibitors at lower biologically active doses, rather than the maximum tolerated oncology dose tested in previous studies.

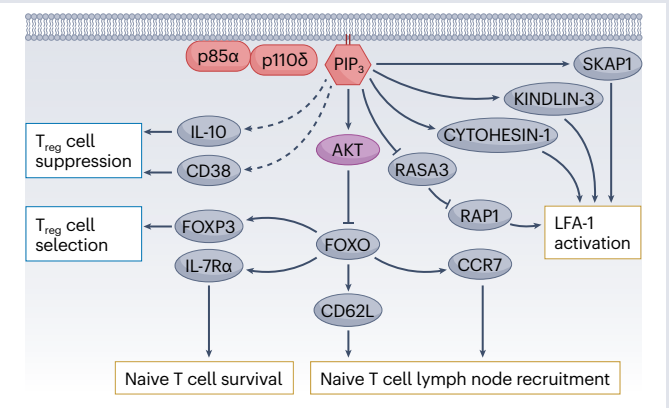
Box 3 | Why does PI3K but not AKT inhibition induce immune-mediated side effects?

$PI(3,4,5)P_3$ (PIP_3)/$PI(3,4)P_2$ have many lipid-binding domain-containing effectors in addition to AKT, including tyrosine kinases (for example, BTK, ITK, TEC), adaptor proteins (for example, DAPP1, GAB1, SKAP1 and KINDLIN-3) and regulators of proteins of the small GTPase superfamily, including GEFs and GTPase-activating proteins (GAPs) of the RAS, RHO, RAB and ARF family. Therefore, AKT and PI3K inhibition do not exert equal effects.

Differences between PI3K and AKT activity affect immune function, particularly T cell function and activity, with evidence of such differential impacts also observed in B cells[345].

AKT inhibition has been shown to induce memory phenotypes in in vitro-expanded conventional T cells for adoptive cell transfer[6,346]; however, a direct comparison of PI3Kδ with AKT inhibition showed that only PI3Kδ inhibitors induce stem memory pathways and a more undifferentiated state[347]. In these cells, PI3Kδ has AKT-dependent and independent effects. PI3Kδ is well established to signal via AKT to inhibit FOXO1-dependent expression of CD62L and CCR7, required for naive T cell recruitment to lymph nodes, and IL-7Rα, for naive T cell survival[348–350] (see the figure). In contrast, PI3Kδ directly activates the PIP_3-binding proteins CYTOHESIN-1, SKAP1, KINDLIN-3 and RASA3 for integrin LFA-1 activation, independent of AKT[350–353] (see the figure).

Regulatory T cell (T_{reg} cell) dysfunction is the major mechanism of action for PI3Kδ inhibition-induced antitumour immune responses and the loss of immune tolerance leading to the major PI3Kδ inhibitor side effects. However, loss of tolerance and immune side effects are not observed upon AKT inhibition, despite a key role for both PI3Kδ and AKT in T_{reg} cells. PI3Kδ has multiple stage-dependent roles in T_{reg} cell development and function. PI3Kδ inactivation in mice enhances T_{reg} cell selection in the thymus but depletes T_{reg} cells in the periphery and reduces the suppressor function of the remaining cells[343]. Mechanistically, thymic T_{reg} cell selection



is dependent on FOXP3 expression, which is negatively regulated by PI3Kδ/AKT signalling and thereby FOXO inhibition[354,355] (see the figure). Consistently, AKT hyperactivation also reduced FOXP3 expression and T_{reg} cell differentiation in the thymus[356]. However, this phenotype is not associated with PI3Kδ inhibitor-induced loss of tolerance, which seems to be driven by reduced peripheral T_{reg} cell suppressor activity[357]. Although the molecular mechanisms by which PI3Kδ promotes T_{reg} cell-mediated suppression are incompletely understood, IL-10 and CD38 production contribute[343,358] (see the figure, dashed arrows indicate indirect regulation of effectors). To the best of our knowledge, no data have shown that AKT promotes T_{reg} cell-mediated suppression via IL-10 and CD38, and transcriptional profiling of PI3Kδ kinase-dead T_{reg} cells revealed no changes in AKT/FOXO target genes compared with wild-type (WT) cells[358], suggesting that PI3Kδ regulates T_{reg} cell-mediated suppression via AKT-independent effectors.

Changes in the regulatory and clinical landscape in haematological malignancies

The development of PI3K inhibitors for haematological malignancies such as CLL occurred in parallel to drug development against BTK and BCL-2. While later-generation BTK and BCL-2 inhibitors are well tolerated, PI3K inhibitors were found to be associated with serious side effects. This led to black box warnings for idelalisib and duvelisib, withdrawal of accelerated FDA approvals for indolent lymphomas, termination or withdrawal of phase III trials and a general pessimism towards targeting PI3Kδ in this disease context[59]. An Oncologic Drugs Advisory Committee (ODAC) meeting convened in 2022 advised that future approvals of PI3K inhibitors in haematologic malignancies should always require a randomized trial using overall survival as the primary end point[60]. This challenge has been further compounded by changes in FDA trial requirements and regulations, including randomized dose-finding studies for all oncology trials[122], an approach which is prohibitive in malignancies with small patient populations. The use of overall survival as an end-point also presents challenges as the life expectancy for patients with CLL or non-Hodgkin lymphoma is long, with multi-year follow-up; outcomes may be influenced by many factors and not truly reflective of the investigational drug impact[60]. The heavy pretreatment of the patients in these trials is also likely to confound the interpretation of overall survival data[60].

Widening the therapeutic impact of current PI3K/AKT/mTOR inhibitors

There are numerous ongoing approaches and opportunities emerging to exploit the potential of existing ATP-competitive PI3K pathway inhibitors.

Reducing adverse effects

On-target adverse effects remain a challenge for FDA-approved PI3K/AKT/mTOR inhibitors and clinical studies are exploring various approaches to mitigate these.

Medications have been used to limit negative side effects. Prophylactic use of metformin reduced the incidence and severity of hyperglycaemia in patients with *PIK3CA*-mutant $HR^+/HER2^-$ breast cancer treated with alpelisib/endocrine therapy, reducing treatment discontinuation[123]. Prophylactic non-sedating (H_1) antihistamine therapy is advised to prevent rashes in patients treated with alpelisib[124]. Furthermore, the phase II SWISH study demonstrated that dexamethasone mouthwash prevents stomatitis in patients with $HR^+/HER2^-$ metastatic breast cancer treated with everolimus[125]. To reduce the risk of opportunistic infections, prophylaxis for *Pneumocystis jirovecii pneumonia*, antiviral therapy to prevent reactivation of varicella zoster virus or herpes simplex virus and monitoring for cytomegalovirus are recommended for patients with leukaemia treated with idelalisib or duvelisib[126,127].

Another strategy that has been explored is the use of alternative routes of administration. Inhaled inhibitors developed for asthma and chronic obstructive pulmonary disease include the PI3Kδ inhibitors nemiralisib (GSK2269557 (GSK))[128] and CHF-6523 (Chiesi)[129,130], and the dual PI3Kγ/δ inhibitor AZD8154 (AstraZeneca)[131]. However, due to lack of efficacy, none of these compounds remains under active development. An example of a topical skin application is the PI3K/mTOR inhibitor bimiralisib in an active phase II trial for actinic keratosis (NCT06319794), with promising results[132]. Another approach aimed at reducing adverse effects has been the use of an esterified prodrug that is inactive in enzymatic assays with purified PI3K proteins but, upon metabolism by esterases in the cytosol, is converted to an active PI3K inhibitor. An example of this is the inhaled prodrug PI3K inhibitor CL27c in preclinical models of asthma and fibrosis[133].

Encapsulation of alpelisib into P-selectin-targeted nanoparticles, allowing dose reductions, has also been explored in preclinical models of head and neck cancer[134].

Dosing considerations

Overall, there appear to be no clear rules for optimal dosing strategies, and instead studies have adapted based on empirical preclinical observations, with the aim of achieving sustained target inhibition while limiting tolerability issues.

For PI3Kα inhibitors, the first-in-human study of alpelisib showed that continuous daily dosing achieved sustained plasma exposure and dose-dependent suppression of pAKT and downstream biomarkers, and correlated with clinical activity[135]. Similarly, FDA pharmacology reviews for inavolisib document exposure–response analyses that support once-daily dosing[136].

By contrast, PI3Kδ inhibitors such as idelalisib, duvelisib and umbralisib were developed as continuous daily or twice-daily oral regimens for indolent non-Hodgkin lymphoma, but this approach was limited by cumulative immune-mediated toxicities. In APDS, by contrast, continuous normalization of PI3Kδ signalling is required, and leniolisib is dosed once daily, with phase III data confirming durable biomarker and clinical improvements[120].

Copanlisib, an intravenous PI3Kα/δ inhibitor, was deliberately developed with an intermittent schedule (days 1, 8 and 15 of a 28-day cycle) to deliver transient high-level target inhibition without drug washout, due to its long half-life, possibly as a result of accumulation of the basic molecule in acidic tumours[137,138]. This was associated with fewer chronic immune toxicities than daily PI3Kδ inhibitors, although it introduced distinct acute infusion-related toxicities such as hyperglycaemia and hypertension due to systemic PI3Kα blockade. More recently, next-generation PI3Kδ inhibitors such as zandelisib have adopted intermittent oral schedules (7 days on/21 days off) to exploit the persistence of pharmacodynamic effects while improving tolerability[139].

Intermittent dosing in combination with intravenous administration is being tested with the pan-class I PI3K/mTOR inhibitor gedatolisib (Celcuity). One hypothesis is that orally administered PI3K inhibitors disrupt gut epithelial barrier integrity, resulting in mucosal immune activation and gut toxicity[82]. Such effects may be overcome by intravenous dosing, with some supportive evidence emerging from a phase Ib breast cancer trial with gedatolisib. Indeed, a regime of weekly intravenous dosing for 3 weeks, followed by 1 week off, appears to induce a lower frequency and severity of hyperglycaemia and diarrhoea than historical observations with oral dosing of this compound, and no grade 3/4 colitis[140].

AKT inhibitors illustrate the same principles: capivasertib employs a 4 days on/3 days off regimen supported by PK/PD data showing robust suppression of pAKT with recovery periods that improved tolerability[141]. Ipatasertib, in contrast, was developed with continuous daily dosing, which achieved acceptable PK/PD but yielded mixed efficacy in phase III trials[142–144].

Collectively, these examples highlight that continuous daily dosing is favoured where sustained suppression is required and tolerated, whereas intermittent regimens are increasingly employed to balance durable pathway inhibition against cumulative toxicities.

Overcoming drug resistance in cancer

Given the lack of direct cytotoxic activity on cancer cells, it will be necessary to combine PI3K/AKT/mTOR pathway inhibitors with other drugs to achieve cell killing. Many combinations have been tested with first-generation PI3K pathway inhibitors, without much success, again partially due to drug tolerability issues.

A key challenge is to uncover cancer contexts in which aberrant PI3K/AKT/mTOR activation creates a vulnerability to a specific combination treatment, as was successfully achieved in $HR^+/HER2^-$ breast cancer where PI3Kα/AKT are key regulators of ER expression and functionality, and thus of hormone sensitivity[46,47].

Complementary tumour drivers also exists in prostate cancer between androgen receptor (AR) responsiveness and PI3K/AKT/mTOR pathway activation (mainly through *PTEN* loss), with *PTEN* loss associated with a poorer response to AR blockade, and PI3K pathway inhibition leading to increased androgen pathway activity[145–147]. AstraZeneca recently reported positive results with capivasertib in combination with abiraterone (androgen biosynthesis inhibitor) and androgen deprivation therapy in the CAPItello-281 phase III trial in *PTEN*-deficient de novo metastatic hormone-sensitive prostate cancer[148]. However, in other combinations for prostate cancer including with endocrine therapy, such as in the RE-AKT (with enzalutamide)[149] or ProCAID (with docetaxel and prednisolone)[150] trials, capivasertib did not increase clinical benefit. The observation that enzalutamide leads to a significant reduction in capivasertib exposer may have impaired the efficacy of the AKT inhibitor in this context. The CAPItello-280 trial testing capivasertib with docetaxel and androgen deprivation therapy has been discontinued as it was unlikely to meet primary end-points[151].

In hormone-independent triple-negative breast cancer (TNBC), the PI3K pathway–hormone signalling interaction is not therapeutically relevant. Even though many TNBCs contain *PIK3CA*/*AKT1*/*PTEN* mutations, the CAPItello-290 trial showed that capivasertib did not improve the efficacy of paclitaxel chemotherapy[152]. However, a novel tissue-specific vulnerability has recently been identified in TNBC with the combination of AKT and the EZH2 histone methyltransferase inhibitors driving basal-like TNBC cells into a more differentiated, luminal-like state, and then inducing cell death by hijacking the same regulatory signals as during luminal cell involution[153].

The observations in prostate cancer and TNBC illustrate the challenges to uncover effective PI3K pathway combinations. In some instances, PI3K pathway inhibition may actually reduce the activity of the combination drug, as has been suggested for mTORC1 inhibitors which — through the induction of cytostasis — provide resistance to cell cycle progression-targeting chemotherapy agents in leukaemic cells[154,155].

Combinations of multiple PI3K/AKT/mTOR inhibitors, described as multi-node inhibition, have also been suggested as an approach to prevent the reactivation of signalling via relief of negative feedback loops and to overcome the presence of mutations in multiple pathway components[156]. Indeed, inhibition of PI3Kα, mTORC1 and mTORC2 with sapanisertib and serabelisib has shown superior anticancer activity in in vitro and in vivo models[156] and early promising results in a phase I trial in combination with paclitaxel[157].

Dietary modifications have also been suggested as a strategy to prevent PI3K pathway inhibitor resistance mechanisms. A ketogenic diet and anti-hyperglycaemic therapy improved the efficacy of PI3K/mTOR inhibitors in preclinical models[158,159] and hyperglycaemia was associated with lower progression-free survival in patients with glioblastoma treated with BKM-120 (ref. 159). These observations were proposed to be due to PI3K inhibitor-induced hyperglycaemia driving hyperinsulinaemia, which further activates oncogenic signalling in cancer cells[158] and is being further tested in patients with glioblastoma and breast cancer treated with PI3K inhibitors and a ketogenic diet and/or anti-hyperglycaemics (NCT05183204, NCT05090358). However, a recent study has claimed that the anti-oncogenic effects of the ketogenic diet in combination with PI3K inhibition, at least in rodent models, are insulin-independent[160]. The murine ketogenic diet is low in soy-derived soyasaponins which are converted by the gut microbiome to soyasapogenols that promote liver cytochrome P450 enzyme expression. As PI3K inhibitors are metabolized by P450 enzymes, the low P450 levels in ketogenic diet-fed mice increase the levels of circulating PI3K inhibitor, thereby enhancing their antitumour effect[160]. It is not clear at present whether ketogenic diet in humans would also affect PI3K inhibitor exposure.

PI3Kδ inhibitors for B cell malignancies, myelofibrosis and immune/inflammatory conditions

As mentioned above, the development of PI3K inhibitors for haematological malignancies has met with serious challenges[59], and there has even been a suggestion by regulators that all PI3K inhibitors and their toxicities should be evaluated as a class, rather than based on their individual characteristics[161]. However, this approach is misinformed, given that well-tolerated inhibitors of PI3Kδ or PI3Kγ can be developed, as illustrated by leniolisib, eganelisib (see below) and data so far for roginolisib.

Some B cell malignancies, such as CLL and FL, remain prime indications for PI3Kδ inhibitors given their dependency on constitutive PI3Kδ-dependent BCR signalling[61,62], creating a specific vulnerability. An unmet clinical need remains in these malignancies for well-tolerated, non-chemotherapy-based approaches, especially in conditions of resistance to current therapies. In this context, it is of interest to note that preclinical data support the potential synergy of roginolisib with the BCL-2 inhibitor venetoclax in BTK inhibitor-refractory CLL[162]. A trial of roginolisib in combination with venetoclax and rituximab in refractory/relapsed CLL has now been initiated (NCT06644183).

Myelofibrosis is a chronic blood disorder belonging to a group of diseases known as myeloproliferative disorders. A trial of roginolisib in combination with the JAK inhibitor ruxolitinib is ongoing (HEMA-MED; NCT06887803).

An important outstanding question is whether roginolisib and leniolisib could also be used in disease conditions resulting from aberrant immune activation, given that PI3Kδ might be a key signalling node in many such deregulated immune contexts. Leniolisib is now also being tested in non-APDS conditions, namely in patients with genetically defined primary immunodeficiency disorders linked to PI3K signalling (NCT06549114) or with common variable immunodeficiency (NCT06897358). At present, the contribution of PI3Kδ activity in these diverse disease settings is unknown and might determine the success of this intervention. These trials will help address the important question on the utility of PI3Kδ inhibitors in chronic inflammatory conditions, given their lack of clinical efficacy in such conditions to date, and the observation that strong, sustained PI3Kδ inhibition may result in immune activation rather than immunosuppression, through a rebalancing of the adaptive immune system.

Alternative approaches to target PI3Kγ function

PI3Kγ was the first PI3K isoform subjected to extensive inhibitor development, with eganelisib (IPI-549 (Infinity)), ZX-4081 (Nanjing Zenshine

Pharmaceuticals; NCT05118841)[163] and HS248 (Hanhui Pharmaceutical Co.; NCT05759234) reaching clinical trials in solid malignancies, based on preclinical evidence that PI3Kγ inhibition potentiates immune checkpoint therapy by dampening immunosuppressive myeloid cells[164,165].

Recent data[166–168] have indicated that PI3Kγ could be a cell-intrinsic target in various leukaemias, particularly acute myeloid leukaemia[169,170]. However, some inconsistencies exist, with one study[168] indicating that kinase inhibitors of PI3Kγ do not recapitulate the anti-leukaemic effects observed by downregulation of *PIK3CG* or *PIK3R5*, which motivated the development of a small molecule PI3Kγ degrader[168] (discussed further below). Stelexis BioSciences has now acquired eganelisib to test it as monotherapy and in combination with cytarabine in acute myeloid leukaemia or high-risk myelodysplastic syndrome (NCT06533761).

A PI3Kγ mimetic peptide known as KIT-2014 (Kither Biotech) has been developed to target the scaffolding function of PI3Kγ, independent of kinase activity, increasing cAMP concentrations in specific microdomains within the cell, and thereby regulating immune cell function, smooth muscle contraction and fluid secretion[171]. KIT-2014 shows promise in preclinical models of chronic obstructive respiratory disease[171] and has entered a phase I trial in healthy participants (NCT06659757).

Cancer immunotherapy of solid tumours

Although immunotherapy has revolutionized cancer treatment, it is only effective in a fraction of patients. There is a need for alternative ways to harness the immune system as an anticancer strategy.

Initial efforts to target cancer cell-extrinsic PI3K focused on PI3Kγ inhibition to dampen immunosuppressive myeloid cells to potentiate immune checkpoint therapy[164,165], with some clinical data currently emerging[40,41]. There might also be an opportunity for PI3Kβ inhibitors in this context, given the evidence that tumour-intrinsic PI3Kβ controls immune evasion in solid tumours with *PTEN* loss[172,173] and the observation that these inhibitors are well tolerated in humans[39,44].

The realization that PI3Kδ inhibition can induce a host anticancer immune response[77,107,108], together with the development of well-tolerated PI3Kδ inhibitors such as leniolisib and, potentially, roginolisib, has prompted further exploration of the potential of PI3Kδ inhibition in cancer immunotherapy. Roginolisib monotherapy has shown promising antitumour activity in patients with uveal and cutaneous melanoma who had progressed on PD1/PDL1 inhibitors[111]. FDA fast-track designation for roginolisib was granted for uveal melanoma in 2023, with a registration trial for this indication now planned (OCULE-01; NCT06717126). A roginolisib immunotherapy trial in metastatic non-small cell lung cancer (NSCLC) in combination with anti-PD1 with or without docetaxel is also planned (PULMO-01; NCT06879717). Linperlisib has also been tested in a phase I advanced solid tumour immunotherapy basket trial showing acceptable tolerability[174].

It would also be of interest to explore the potential of leniolisib in cancer immunotherapy.

Rare diseases associated with genetically driven PI3K/AKT/mTOR pathway activation

Aberrant genetic PI3K/AKT/mTOR activation, due to activating mutations in pathway components or inactivating mutations in negative regulators, causes a group of rare diseases, sometimes referred to as TORopathies[175].

The EPIK-P2 trial reported that alpelisib is active and well tolerated in a small fraction of patients with PROS[13,63]. A total 16.7% and 23.2% of adult and paediatric patients, respectively, demonstrated an objective response (defined as a ≥20% reduction from baseline in the sum of target lesion volumes of one to three lesions) and 57.4% of adult patients experienced adverse effects[54]. The FDA approval of alpelisib for PROS was based on the EPIK-P1 retrospective real-world trial, with 37.5% of patients exhibiting a ≥20% reduction in target lesion volume(s) with additional clinical benefits observed across the whole patient population[176]. Given the severity of PROS, the lack of alternative approved targeted therapies and the clinical benefit in many patients (even if many did not meet the definition of an objective response), the risk–benefit ratio was in favour of alpelisib approval for PROS; however, additional prospective studies are required to fully assess the benefits[54,176]. A small study in paediatric patients recently showed that a greater efficacy with reduced adverse effects may be achieved by age or weight-based alpelisib dosing[177]. In these patients, mutant-selective PI3Kα inhibitors may be more therapeutically beneficial and exhibit reduced toxicity (see below). The pan-AKT inhibitor miransertib has also shown short-term positive outcomes and tolerability in small studies of patients with PROS or Proteus syndrome[178,179] and a larger clinical trial is ongoing for Proteus syndrome (caused by *AKT1* mutations; NCT04316546).

Somatic mosaic mutations in the PI3K/AKT/mTOR signalling pathway are often associated with vascular malformations[180], and in cases with other underlying genetic causes PI3K/AKT/mTOR pathway activation is often a characteristic feature, as in, for example, hereditary haemorrhagic telangiectasia and Klippel–Trenaunay syndrome[181]. Rapalogs are commonly used in vascular malformations. Early positive clinical trial proof-of-concept data in hereditary haemorrhagic telangiectasia were recently announced for engasertib (also known as VAD044), an oral allosteric AKT inhibitor (Vaderis Therapeutics/Almac)[182], which was also granted FDA fast-track designation for this indication.

Other syndromes associated with PI3K/AKT/mTOR activation that might benefit from inhibitors of this pathway include PTEN hamartoma tumour syndrome[183] (due to heterozygous germline inactivation of *PTEN*, causing macrocephaly and autism spectrum disorder and predisposing to specific types of solid tumours), SHORT syndrome (*PIK3R1* mutations)[184], megalencephaly–polymicrogyria–polydactyly–hydrocephalus syndrome (*PIK3R2* or *AKT3* mutations)[185], Smith–Kingsmore syndrome and focal cortical dysplasia type II (*MTOR* mutations)[186].

Novel pharmacological approaches to modulate PI3K/AKT/mTOR signalling

New chemical approaches and increased structural insight, for example the discovery of druggable allosteric pockets, are currently being exploited to target PI3K/AKT/mTOR pathway components beyond the classical reversible ATP-competitive inhibitors (Fig. 2).

Conformation-selective inhibitor

Roginolisib represents the first example of a new class of PI3Kδ inhibitors[187]. Despite binding at the orthosteric ATP site, roginolisib is non-competitive with ATP[118]. Structural studies, including X-ray crystallography and hydrogen–deuterium exchange mass spectroscopy, coupled with molecular dynamics simulations, were used to investigate the binding mode and these demonstrate that the compound produces effects on the PI3Kδ protein beyond the ATP site[187]. Idelalisib and related 'propeller'-shaped inhibitors bind to p110δ where movement of Met752 opens a 'selectivity pocket' that favours inhibition of PI3Kδ (refs. 188,189). Roginolisib, in contrast, does not have a propeller

shape and does not access this pocket, but is instead bound in an almost planar conformation which is accommodated by the enzyme adopting an inactive conformation where there is a change in the position of the kα12 helix. This conformation has not been observed with previous PI3Kδ inhibitors and is disfavoured in other isoforms, particularly PI3Kα and PI3Kγ that lack two basic residues, Lys1040 and Arg1043. The molecular dynamics studies show that binding of roginolisib to PI3Kδ induces a change in the conformation of Asp911, which together with Asp897 then forms electrostatic interactions with Lys1040 and Arg1043. It is hypothesized that these differences in binding mode and the resultant excellent cellular PI3K isoform selectivity translate into the improved clinical safety observed for roginolisib. Treatment at a biologically active dose, rather than the maximum tolerated dose, may also contribute to the improved safety profile. This improved safety, in turn, has enabled long-term dosing of patients without requirements for dosing holidays in early-phase trials[82,111,118].

Mutant-selective inhibitors

The current FDA-approved PI3Kα inhibitors (alpelisib and inavolisib) target both wild-type (WT) and mutant PI3Kα (refs. 190,191). Inhibition of WT PI3Kα in healthy cells is the most likely reason for toxicities observed with these compounds, most notably hyperglycaemia and diarrhoea. Drugs that inhibit mutant PI3Kα, while sparing PI3Kα-WT, are expected to maximize both efficacy and tolerability in *PIK3CA*-mutant cancer[4]. However, it is possible that these compounds will also be subject to some of the same resistance mechanisms that hinder the long-term efficacy of other classes of PI3K inhibitors, including the selection for mutations in downstream pathway components.

An exciting development is that *PIK3CA* mutant-selective compounds have been generated, which are either pan-mutant PI3Kα inhibitors or target the oncogenic hot-spot variant PI3Kα-H1047R. These include STX-478 (also known as tersolisib and LY4064809; Scorpion Therapeutics)[192], RLY-2608 (also known as zovegalisib; Relay Therapeutics)[193] and LOXO-783 (Lilly)[194]. These compounds bind outside the ATP-binding site and allosterically target a cavity which is more accessible in the PI3Kα-H1047R mutant than in PI3Kα-WT (refs. 192,195,196). Preclinical characterization of several additional PI3Kα mutant-selective inhibitors has recently been reported, in some cases with improved efficacy and specificity compared with STX-478, including CGT6297 (Cogent Biosciences)[197], LAE118 (Laekna Therapeutics)[198], GSC002639 (Changchun GeneScience Pharmaceutical)[199], TY-2291 (TYK Medicines)[200] and REC-7735 (Recursion). Clinical trials are in progress with STX-478, RLY-5836 and RLY-2608 (ref. 201), whereas the development of LOXO-783 was discontinued. In January 2025, Lilly acquired the STX-478 programme from Scorpion Therapeutics. Early evidence shows enhanced drug tolerability compared with pan-PI3Kα inhibitors, without loss of clinical impact, as detailed below.

The first in-human results of STX-478 in patients with advanced solid tumour were reported by Scorpion Therapeutics at the end of 2024 (ref. 202). The report showed that STX-478 was well tolerated with no hyperglycaemia at the maximum tolerated dose of 100 mg daily, without any of the significant grade 3 rash, mucositis or diarrhoea that is observed with other PI3K inhibitors, but with mild-to-moderate fatigue as an adverse effect. STX-478 was active in breast and non-breast cancers, with an overall response rate exceeding historical comparisons with other PI3K inhibitors. Moving forward, STX-478 in combination with CDK4/6 inhibitors and fulvestrant is being studied in first-line and second-line $HR^+/HER2^-$ breast cancer with *PIK3CA* mutations (NCT07174336).

RLY-2608 in combination with fulvestrant is showing early promising results in the first-in-human ReDiscover trial in *PIK3CA*-mutant, $HR^+/HER2^-$ breast cancer with a favourable safety profile compared with currently FDA-approved PI3K pathway inhibitors[201]. Interestingly, in patients with advanced breast cancer, acquired resistance to the PI3Kα-selective inhibitors alpelisib and inavolisib was recently shown to involve *PTEN* loss[105], activating *AKT1* mutations or secondary mutations in the *PIK3CA* catalytic pocket that alter inhibitor binding[203]. Cell-based studies indicated that all of these secondary *PIK3CA* mutations could be overcome by RLY-2608 (ref. 203). The RLY-2608 + fulvestrant combination progressed in 2025 to a phase III trial versus capivasertib + fulvestrant in patients with $HR^+/HER2^-$ breast cancer treated with CDK4/6 inhibitors and endocrine therapy (NCT06982521). RLY-2608 is also being tested in a phase II study in adult and paediatric patients with PROS[204] (NCT06789913).

Crystallographic and molecular dynamics studies have shown that an effect of the H1047R mutation is to change the dynamics of the carboxy-terminal region of the protein[195]. In a cryo-electron microscopy structural analysis, the C-terminal chain extending from H1047 adopts a defined position in WT PI3Kα, whereas in the mutant this region is disordered and not visible.

One group of p110α mutant-selective inhibitors, including Q-7909, have been shown by cryo-EM to bind in an allosteric pocket that is close to H1047 in mutant p110α (ref. 205) (Fig. 4). The carboxylate group on the inhibitor induces a reorientation of the terminal guanidine of R1047 to make a charge-charge interaction (Fig. 4).

RLY-2608 and STX-478 bind in a different allosteric pocket of PI3K (ref. 206) that results from movement of the activation loop. Both compounds form hydrogen bonds with L911; however, the activation loop adopts different conformations for the two different molecules, with hydrogen bonds forming between L941 and STX-478 and between D1018 and RLY-2608 (Fig. 4). This pocket is not adjacent to H1047 and no direct interaction is formed with the mutant, but destabilization of the conformation of the C terminus of p110α makes the pocket more accessible in the H1047R mutant than in WT. The allosteric pocket is accessible in WT PI3Kα, but the additional energy required to displace the C-terminal region and rearrange other residues likely explains the selectivity observed for mutant PI3Kα with compounds that bind in this pocket. The allosteric pocket is relatively close to the ATP-binding site and structural changes that occur upon formation of this pocket account for the observed inhibition of the enzyme.

However, these compounds have fared differently in the clinic, with RLY-2608 showing activity only in combination with anti-oestrogen in ER^+ metastatic breast cancer[207], whereas STX-478 demonstrated monotherapy activity in ER^+ metastatic breast cancer and other *PIK3CA*-mutant cancer types[202]. At present, it is not clear whether the conformational differences drive the different clinical profiles seen for STX-478 and RLY-2608.

Although the prospect of a tumour-agnostic approval for PI3K/AKT inhibitors in any *PIK3CA*-mutant tumours remains improbable, basket trials are ongoing and showing some modest success[143] and mutant-selective inhibitors appear the most promising drugs for a basket clinical trial concept in other cancer types. This particularly applies to STX-478 given its activity as monotherapy.

Covalent inhibitors

In general, covalent kinase inhibitors exhibit increased inhibitor potency compared with reversible inhibitors, provide longer duration of inhibition and provide the potential for increased selectivity amongst

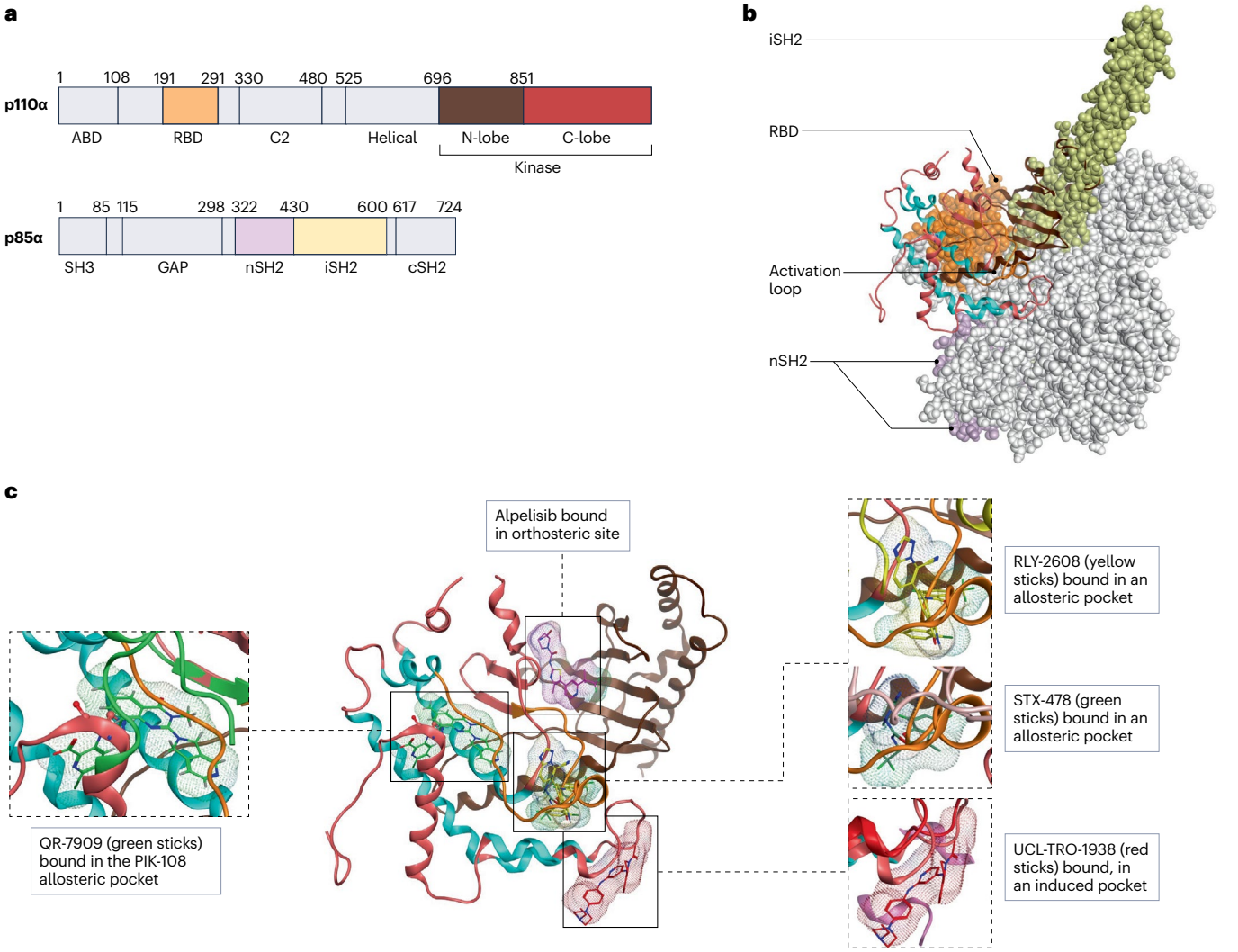


Fig. 4 | Allosteric PI3Kα inhibitor binding sites. a, Domain organization of PI3Kα. **b,c**, Crystal structure of PI3Kα (4JPS), solved bound to alpelisib (not shown in panel **b**) at the ATP site. Structure of the p110α subunit, including the RBD (light brown spheres), amino-terminal domain (dark brown), carboxy-terminal domain (salmon), helices of regulatory arch (light blue), activation loop (orange) and kinase domain (ribbons), H1047 (spheres and sticks) and other domains (white spheres), and the p85 subunit, including nSH2 (purple) and iSH2 (yellow) (panel **b**). The kinase domain showing the orthosteric site with alpelisib (purple sticks) bound, two allosteric inhibitory sites with QR-7909 (green sticks), RLY-2608 (yellow sticks) and STX-478 (green sticks) bound, and the site where the allosteric activator UCL-TRO-1938 (red sticks) binds. QR-7909 binds the PIK-108 allosteric pocket: activation loop and C-terminal chain (green ribbons), R1047 (green sticks with spheres) (in contrast to H1047 of 4JPS (salmon sticks and spheres)). RLY-2608 binds in an allosteric pocket: activation loop (where resolved) (yellow). STX-478 binds in an allosteric pocket: activation loop (where resolved) (pale pink ribbon). UCL-TRO-1938 binding showing the shifts of C-terminal protein chains (red ribbon); the fit with the chain of the C2 and helical domains that form the other wall of the induced pocket are shown (dark pink ribbons) (panel **c**).

related kinases[208–210]. For example, wortmannin, one of the first identified PI3K inhibitors, inactivates multiple PI3K isoforms by covalent modification of an active-site catalytic Lys in the ATP-binding site[211].

The first rationally designed covalent PI3Kα inhibitor was reported in 2013 (CNX-1351; Celgene Avilomics Research)[212]. Further examples have been reported more recently, including compounds 19 and 22 reported by the Wymann group[213], their more advanced compound 9 (ref. 95) and TOS-358 (chemical structure undisclosed; Totus Medicines)[214]. The disclosed molecules consist of existing reversible ATP-competitive inhibitor scaffolds in which an electrophilic reactive functional group ('warhead') is incorporated to engage in a covalent interaction with PI3Kα, to induce irreversible kinase inhibition. These compounds target the non-catalytic Cys862 in the p110α kinase domain that is not present in the other PI3K isoforms, inhibiting both WT and mutant PI3Kα (refs. 95,212,213).

In *PIK3CA*-mutant cell lines, compound 9 exerted a superior in vitro anti-proliferative effect over alpelisib and inavolisib[95]. This was not the case in cell lines lacking functional PTEN, in which persistent PI3Kα inhibition was rapidly bypassed by other class I PI3K isoforms[95]. The anti-proliferative effect of compound 9 correlates with G1 cell cycle arrest without induction of cell death[95], as measured by growth rate inhibition metrics[83,84]. Interestingly, compound 9 was also found,

similar to inavolisib, to induce substantial p110α protein degradation in cell lines[95].

TOS-358 has also been reported to have superior efficacy compared with reversible PI3Kα inhibitors, and to induce potent, selective and durable PI3Kα inhibition without significant hyperglycaemia in animal models[214]. The reason for the lack of hyperglycaemia remains unclear. However, it may be due to compensation by PI3Kβ, which would be facilitated if covalent inhibitors also lead to p110α protein degradation, allowing PI3Kβ to become recruited to binding sites in signalling complexes normally occupied by PI3Kα. TOS-358 has recently entered clinical trials in solid tumours (NCT05683418)[215].

Covalent inhibitors of PI3Kδ have also been generated, by providing an ATP-competitive PI3Kδ inhibitor with the functionality to acetylate the catalytic Lys779 (refs. 216–218). As acetylation is a naturally occurring post-translational modification it is thought that this approach would mitigate the risk of immune-mediated adverse effects caused by exogenous protein modifications induced by other covalent inhibitor approaches[216]. There is also some evidence to suggest that catalytic Lys residues may be less prone to resistance-inducing mutation compared with reactive Cys residues proximal to the active site, the target of many covalent inhibitors[216]. An additional series of Lys779-targeting alaninamide-containing covalent PI3Kδ inhibitors have been developed, with the optimized compound forming an amide bond with Lys779 and showing anti-proliferative effects in haematopoietic cell lines[219].

An allosteric covalent AKT inhibitor targeting the mutant Lys in the most common E17K mutation in AKT1 was recently reported, with selectivity for AKT1-E17K over AKT2 and AKT1-WT and no induction of hyperglycaemia in AKT1-E17K tumour xenograft models[220,221].

Weisner et al. generated an allosteric PH domain-dependent AKT inhibitor that covalently reacts with non-catalytic cysteines in AKT (Cys296 and Cys310 in AKT1, conserved in AKT2 and AK3)[222]. This inhibitor not only binds the cysteines but also interacts with a kinase–PH domain interphase to stabilize an inactive conformation.

A covalent inhibitor of mTOR (DHM25) has also been reported[223]. It is hypothesized that inhibition of both mTORC1 and mTORC2 using this covalent inhibitor will overcome the resistance issues associated with mTORC1-specific inhibitors via relief of negative feedback loops.

Degraders

In addition to functions dependent on their catalytic activity, some PI3Ks are known to have protein scaffolding functions[18,19,224]; PI3K degradation may therefore have a stronger and different functional impact to inhibitors that only block catalytic activity. Degraders are molecular glues that bring target proteins in contact with the cellular protein degradation machinery. Proteolysis targeting chimeras (PROTACs) have been generated for several PI3K/AKT/mTOR pathway components, all of which are at the preclinical stage. These include degraders of PI3Kα (refs. 225–227), PI3Kβ (ref. 228), PI3Kγ (ref. 168), PI3Kδ (ref. 229), AKT[230–235], mTOR[236] or dual PI3K/mTOR (ref. 237). Degraders of AKT[230–233] or PI3Kγ (ref. 168) have shown superior inhibition of proliferation and growth inhibition compared with the corresponding ATP-competitive inhibitors.

Interestingly, some non-PROTAC-based PI3K inhibitors also induce p110α protein degradation in cell lines. These include the ATP-competitive reversible inhibitors inavolisib and taselisib (GDC-0032)[190,191] and the covalent PI3Kα inhibitor compound 9 (ref. 95). A32 is a recently reported additional PI3Kα inhibitor that induces degradation of the p110α-H1047R mutant[238].

The protein degradation function of these compounds was serendipitous and the molecular mechanism is unclear. This phenomenon has been best studied with inavolisib and taselisib, which preferentially degrade mutant PI3Kα in cells, in a ubiquitin-dependent and proteasome-dependent manner, despite inhibition of WT and mutant PI3Kα to the same extent in in vitro biochemical assays[190,191]. This inhibitor-induced PI3Kα-mutant degradation is context-dependent[226] and is most clearly observed in the membrane fraction of cells; in cells with high RTK activity, especially in HER2-amplified cancers; and when p110α is in complex with the p85β regulatory subunit[190,191]. The mechanism of endogenous p110α turnover is unknown. The p85 subunits are known to bind the c-cbl E3 ubiquitin protein ligase, with some reports indicating preferential binding of p85β over p85α in specific contexts[239]. p85α and p85β have also been reported to associate with the FBXL2 F-box protein, the substrate receptor component of the SCF (Skp1–Cullin–F-box) E3 ubiquitin protein ligase, with only p85β being targeted for proteolysis by this complex[240]. It is possible that conformational changes in PI3Kα, facilitated by recruitment to receptor complexes, expose sites for ubiquitination promoting proteasomal degradation. As oncogenic *PIK3CA* mutations mirror the confirmational changes that occur during WT p110α activation, the mutants may be in a more open and/or membrane-bound configuration, and therefore more susceptible to ubiquitination.

In the context of PI3K degraders, it is important to consider the scaffolding function of these isoforms and how evidence from mouse PI3K gene targeting studies indicates that upon loss of one class I PI3K isoform, other isoforms can engage the upstream receptors and compensate[241,242]. This may be beneficial in preventing on-target toxicities such as PI3Kα inhibition-induced hyperglycaemia, whereby PI3Kβ may compensate[242,243]. However, such compensation may also limit drug efficacy.

mTOR inhibitors

Rapamycin and rapalogs are first-generation mTOR inhibitors that predominantly inhibit mTORC1. This allosteric inhibition is based on the binding of rapamycin to the ubiquitously expressed FKBP12 protein. The rapamycin/FKBP12 complex then interacts with the FKBP–rapamycin binding (FRB) domain in mTORC1 and inhibits the enzyme activity towards S6K.

Second-generation mTOR inhibitors are orthosteric ATP-competitive kinase inhibitors (sometimes called TORKinibs) that target both mTORC1 and mTORC2. It is not clear whether second-generation mTOR inhibitors are currently undergoing clinical development.

RapaLinks (also called bitopic or bisteric inhibitors) are third-generation mTOR inhibitors in which rapamycin is chemically linked in a single molecule to an ATP-competitive mTOR kinase inhibitor, thereby interacting with both the FRB domain and the ATP-binding site in mTOR, to target the mTORC1 substrates S6K and 4E-binding protein-1 (4E-BP1), while sparing mTORC2 (refs. 64,244–246). Rapalogs continue to be under further clinical development, and the bisteric inhibitor of mTORC1 RMC-5552 (Revolution Medicines)[247] has shown early signs of activity with a tolerable safety profile in a phase I clinical trial (NCT04774952, NCT05557292)[248].

Dual PI3K/mTOR inhibitors

ATP-competitive pan-class I PI3K/mTOR inhibitors, such as gedatolisib (Celcuity) and bimiralisib (Torqur), have been designed to block multiple nodes in the pathway. A phase III study testing gedatolisib + palbociclib

and fulvestrant as second-line treatment in patients with HR$^+$/HER2$^-$ advanced breast cancer who have progressed after first-line CDK4/6i treatment (VIKTORIA-1; NCT05501886) is ongoing. This trial has a complex design, with three arms each for patients with *PIK3CA*-WT tumours and also *PIK3CA*-mutant tumours. Notably in the arm of patients with *PIK3CA*-mutant tumours, alpelisib + fulvestrant is a comparator arm, pitting a dual PI3K/mTOR inhibitor against a PI3Kα inhibitor. To date, the gedatolisib + palbociclib + fulvestrant triplet and gedatolisib + fulvestrant doublet promote a clinically meaningful increase in progression-free survival compared with fulvestrant alone in patients with *PIK3CA*-WT tumours[249,250]. Additional trials in the first-line setting in HR$^+$/HER2$^-$ advanced breast cancer (VIKTORIA-2; NCT06757634) are planned and underway in prostate cancer and other solid tumours.

In addition, bimiralisib is being tested as monotherapy with topical skin application for actinic keratosis (NCT06319794) and is showing promising results[132].

Bifunctional PI3K inhibitors

Existing PI3K inhibitors have been conjugated to other pharmacophores that block other cancer-relevant targets, exemplified by bifunctional PI3K/MEK (ref. 251), PI3K/BRD4 (ref. 252), PI3Kδ/BET (ref. 253), PI3K/HDAC (ref. 254) or mTOR/MEK[255] inhibitors. These compounds represent early-stage proof-of-concept efforts, and it remains uncertain whether further drug optimization and their combined on-target effects will allow advancement into clinical development. This combinatorial design of chemotypes to enhance therapeutic impact in cancer will not be described in detail here.

RAS–PI3Kα breakers

PI3Kα binds RAS and is one of its downstream effectors, contributing to the maintenance of RAS-driven cancers[256–258]. The structural basis of the RAS–PI3Kα interaction has recently been reported[259,260], as have screens for small molecules or peptides that block the RAS–PI3Kα interaction[261–263].

Two companies have developed small molecules that, through covalent ligation of Cys242 in PI3Kα, a residue adjacent to its RAS-binding interface, disrupt the binding of PI3Kα to KRAS, HRAS and NRAS. This Cys242 is unique to PI3Kα and not present in other class I PI3K isoforms. The RAS–PI3Kα breakers do not inhibit PI3Kα kinase activity, but act on both WT and helical domain oncogenic hot-spot mutant (E545K) PI3Kα and slow the growth of RAS-driven xenografts in mice, without impacting blood glucose regulation[264,265]. BBO-10203 (BridgeBio Oncology)[264] is now being trialled in cancer (BREAKER-101; NCT0662577), and VVD-699 and related compounds (Vividion Therapeutics)[265] are currently at the phase I trial stage (NCT06804824). A RAS–PI3Kα breaker (FMC-242 (Frontier Medicines)) that inhibits oncogenic RAS signalling and exhibits in vitro efficacy has also been recently developed, but no structural details have yet been disclosed[266].

BBO-10203 was developed via chemical modification of a PI3K-activating molecular glue (D927; described below)[264]. The D927 scaffold was extended towards Cys242 and a suitable electrophilic group added which provides a covalent interaction between BBO-10203 and PI3Kα (Fig. 5). The Vividion compounds bind in a similar induced pocket to BBO-10203 and induce changes to the conformation of helix 4 that destabilizes the interaction with RAS[265] (Fig. 5).

Vividion reported that, compared with RAS mutant tumours, HER2-overexpressing tumours are more consistently and profoundly sensitive to VVD-699 (ref. 265). Unexpectedly, this was also observed with covalent ligands of Cys242 in PI3Kα that do not disrupt its interaction with RAS. These data indicate that the p110α RAS-binding domain also has a currently unidentified role in HER2 signalling, independent of RAS binding, that is disrupted by molecules binding PI3Kα(Cys242)

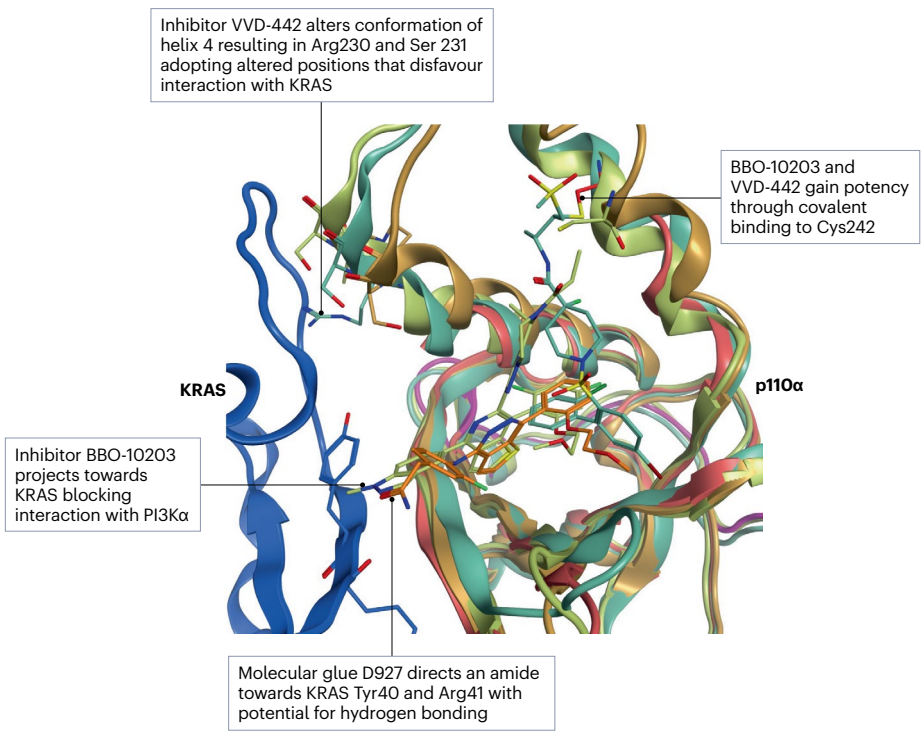


Fig. 5 | Small molecule interactions with the induced allosteric pocket in the RBD of PI3Kα. R4JPS reference (note, region after Lys 228 not observed in 4JPS) (red), 9C15 (ochre) with molecular glue activator D927 (orange sticks), 94BU (yellow) with covalent inhibitor BBO-10203 (yellow sticks) and 9E8M (teal) with covalent inhibitor VVD-442 (teal sticks). KRAS 9C15 shown in blue.

(ref. 265). BridgeBio Oncology also found HER2-amplified tumour lines to be prime responders to BBO-10203 (ref. 264).

PI3K/AKT/mTOR activators

There is ample indirect evidence for therapeutic opportunities by activating PI3K/AKT/mTOR signalling, including in tissue regeneration and protection[267–270]. This includes protection from ischaemia–reperfusion injury upon reoxygenation, such as in neurons following a stroke[271,272] and cardiomyocytes following myocardial infarction[273], protection from ionizing radiation[274], enhancement of tissue/wound repair[267,275] and neuro-protection/regeneration[276–279]. Early discovery work using inhibitors of the lipid phosphatases PTEN, SHIP1 and SHIP2 provided pharmacological evidence that activating the PI3K pathway could have therapeutically beneficial effects[280–283]. However, these reagents are mostly tool compounds, with some efficacy and specificity issues[281,284,285], and although efforts in this area continue[286,287], attention is shifting towards directly activating the kinases in this pathway.

PI3Kα activation. UCL-TRO-1938 is a direct allosteric activator of PI3Kα which has been shown in rodent models to protect from cardiac ischaemia–reperfusion injury and to promote nerve regeneration[288]. Emerging evidence suggests that activators of other PI3K family members can also be generated[289]. UCL-TRO-1938 binds at the interphase of multiple domains in p85/p110α in a pocket that is not present in the PI3Kα apoprotein[288] (Fig. 4). The p85 regulatory subunit keeps p110α in an inactive and stable cytosolic configuration in unstimulated cells, with de-inhibition by recruitment of the p85α/p110α complex to RTKs, adapter proteins or RAS at the plasma membrane[10]. UCL-TRO-1938 induces multiple structural changes compatible with PI3Kα activation, mimicking several steps in the natural activation process of PI3Kα including disruption of inhibitory contacts between p85 and p110α; repositioning of the kinase activation loop; and bringing the ATP loop and the N-lobe of the kinase domain towards the ATP-binding pocket[288].

BridgeBio have identified molecular glue compounds (D927 and D223) that stabilize the RAS–PI3Kα interaction[290]. D927 acts in vivo as a functional mimetic of insulin by dramatically increasing the affinity of RAS for PI3Kα and may provide an insulin-independent means to control blood glucose. In rodent diabetic and obesity models, D927 reduced hyperglycaemia, improved glycaemic control and lowered blood glucose levels in WT animals. These compounds bind in an induced allosteric pocket in the RBD of PI3Kα and modify the binding interaction with RAS (Fig. 5).

AKT activation. The AKT activator SC79 is the first-reported direct small molecule activator of the PI3K pathway, first used in mice to rescue ischaemia-induced neuronal death[291]. SC79 binds to the PH domain of AKT, with SC79-bound AKT possibly adopting a conformation favourable for activating phosphorylation of the enzyme by upstream protein kinases[291]. This tool compound has been used in numerous studies to uncover a positive role for AKT activation in cell protection and tissue regeneration[292–295].

mTORC1 activation. NV-5138 (Navitor Pharmaceuticals; also known as SPN-820 (Supernus Pharmaceuticals)) is an indirect activator of mTORC1 (ref. 296), currently in phase II trials for treatment-resistant depression (NCT05066672)[297]. NV-5138 is a leucine analogue that binds the leucine-binding pocket of Sestrin2 (ref. 296). The latter is a leucine sensor that upon binding to GATOR2 inhibits the GATOR1 complex[298], which is a negative regulator of mTORC1. Binding of leucine by Sestrin2 leads to dissociation of Sestrin2 from GATOR2, allowing the latter to inhibit the negative mTORC1 regulator GATOR1, thus resulting in activation of mTORC1 signalling[298]. The use of NV-5138 in the context of depression is based on the detection of reduced mTORC1 activity in the brain of patients with depression[299].

Conclusion

The PI3K/AKT/mTOR pathway is one of the most frequently aberrantly activated pathways in cancer with important roles in immunity and genetic diseases. To exploit its cancer cell-intrinsic activity, PI3K/AKT/mTOR inhibitors will require combination therapy to achieve a cytotoxic rather than cytostatic anticancer response. The emerging potential of well-tolerated newer PI3Kδ inhibitors will hopefully harness their immunomodulatory anticancer potential. Improved understanding of disease mechanisms and a new generation of pharmaceuticals may unlock the full therapeutic potential of targeting this pathway.

Published online: 30 March 2026

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Acknowledgements
Personal funding for S.E.C. was from European Union H2020-MSCA-IF-2018 GA:838559. Research in the B.V. laboratory was supported by the BBSRC (BB/W007460/1, BB/R017972/1, BB/I007806/1), Cancer Research UK (C23338/A15965, C23338/A25722, DRCRPG-Nov24/100006) and PTEN Research (UCL-16-001, UCL-20-001). J.R.B. acknowledges funding from the National Institutes of Health (NIH) National Cancer Institute (NCI) (R01CA258924) and from the US Department of Defense (grant CA230180). Research in the N.V. laboratory is supported by the NIH/NCI (K08CA245192, DP2CA290245). The authors thank K. Okkenhaug for critically reviewing parts of this Perspective. The authors apologize to any scientists whose studies were unintentionally not cited in this article.

Author contributions
S.E.C. and B.V. wrote the manuscript with contributions from N.V., J.R.B. and M.W.D.P. All authors reviewed and edited the manuscript.

Competing interests
B.V. is a consultant for iOnctura (Geneva, Switzerland); a shareholder of Open Orphan and Poolbeg Pharma (Dublin, Ireland); and a director of S&B Scientific. J.R.B. has served as a consultant for Abbvie, Acerta/AstraZeneca, Alloplex Biotherapeutics, BeiGene, Bristol-Myers Squibb, EcoR1, Galapagos NV, Genentech/Roche, Grifols Worldwide Operations, InnoCare Pharma Inc, Loxo/Lilly, Magnet Biomedicine, Merck and Pharmacyclics; received research funding from BeiGene, Gilead, iOnctura, Loxo/Lilly, MEI Pharma, Nagoon Therapeutics and TG Therapeutics; and serves on the Data Safety Monitoring Board for Grifols Therapeutics. N.V. reports consulting for Apertor Therapeutics, Avenzo Therapeutics, GLG, Guidepoint Global, Reactive Biosciences and Scorpion Therapeutics; royalties from Wolters Kluwer; and stock in Reactive Biosciences, Heligenics, Inc. M.W.D.P. is an employee of and stockholder in AstraZeneca. S.E.C. has no interests to declare.

Additional information
Peer review information *Nature Reviews Drug Discovery* thanks Klaus Okkenhaug and the other, anonymous, reviewers for their contribution to the peer review of this work.

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