



Review

PDK1 signaling in cancer: Current status and future perspectives

Darshana Ballagere Honnasiddappa^a, Mohankumar Ramar^b, Tincy Biju^c, Mallikarjun Sajjan^a,
Chidananda Venkatesh^c, Nayan Kumar Mahadeva^d, Selvaraj Kunjiappan^e,
Damodar Nayak Ammunje^{a,*}, Parasuraman Pavadai^{c,*}

^a Department of Pharmacology, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru, 560054, Karnataka, India

^b Department of Pharmacology, Sir Issac Newton College of Pharmacy, Pappakovil, Nagapattinam, 611 102, Tamil Nadu, India

^c Department of Pharmaceutical Chemistry, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru, 560054, Karnataka, India

^d Department of Pharmacognosy, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru, 560054, Karnataka, India

^e Center for Computational Biology, SRM Institute of Science and Technology, Tiruchirappalli, Tiruchirappalli, 621105, Tamil Nadu, India

ARTICLE INFO

Keywords:

PDK1
Oncogene
cancer signaling
PI3K/AKT pathway
Drug resistance

ABSTRACT

Dysregulation of signaling pathways governing cell growth, survival, metabolism, angiogenesis, invasion, metastasis, and therapeutic response drives cancer progression. The AGC family of kinases, such as AKT, S6K, SGK, RSK, and PKC, is important for downstream signaling. The 3-phosphoinositide-dependent protein kinase-1 protein (PDK1), encoded by the PDK1 gene, phosphorylates activation-loop residues in these kinases and links phosphoinositide signaling to cellular growth, survival, metabolism, and transformation. The structure, regulation, cancer-associated functions, and therapeutic targeting of PDK1 are critically reviewed, with a focus on the pleckstrin homology domain, PIF pocket-mediated substrate docking, the kinase domain, and reported non-catalytic interactions. Experimental results from breast, lung, gastric, liver, hematological, and melanoma models suggest that dysregulated PDK1 signaling can increase the output of the PI3K-AKT-mTOR pathway, invasion, metabolic adaptation, and resistance to specific hormonal, chemotherapeutic, and targeted therapies. In some preclinical models, genetic suppression of PDK1 using siRNA, shRNA, or CRISPR-based methods has been shown to slow tumor cell growth and restore drug sensitivity. However, PDK1 remains an unvalidated therapeutic target in the clinic. There is no selective PDK1 inhibitor that has been clinically approved, and most pharmacological candidates are based on biochemical and cellular data. There is limited genetic evidence and *in vivo* evidence, and no validated clinical evidence specific to PDK1. Emerging PIF-pocket ligands, dual-site inhibitors, allosteric modulators, and protein-degradation strategies are therefore investigational strategies that need to be improved in terms of selectivity, pharmacokinetic characterization, tumor-context validation, and biomarker-guided evaluation.

1. Introduction

The 3-phosphoinositide-dependent protein kinase-1 protein (PDK1), encoded by the PDK1 gene, is a constitutively catalytically competent serine/threonine kinase that regulates multiple AGC-family kinases. The expression and/or amplification of PDK1 and the PDK1 signaling pathway have been reported in several solid tumors and hematological malignancies, which offers a biological rationale for investigating PDK1 as a therapeutic target, as shown in Fig. 1. But there is no selective PDK1 inhibitor that has been clinically approved [1]. Genetic and pharmacological evidence has suggested that PDK1 plays a role in tumor-cell survival, metabolic adaptation, epithelial-to-mesenchymal transition,

invasion, metastasis, and resistance to certain chemotherapeutic, targeted, endocrine, and radiation therapies [2]. However, these results are primarily based on biochemical, cellular, genetic, and animal models, and their clinical applicability is not yet clear. At this time, there is no single known reason for the lack of clinically approved selective PDK1 inhibitors. The relative importance of incomplete understanding of tumor-context dependency, substrate-specific regulation, compound selectivity, pharmacokinetic exposure, compensatory signaling, and biomarker selection is not yet clinically proven [3]. Non-catalytic or scaffolding roles have also been reported for PDK1, which could also impact the output of PDK1 signaling, but which have not yet been investigated for their role in the performance of clinical inhibitors [4].

* Corresponding authors.

E-mail addresses: superdamu@gmail.com (D.N. Ammunje), pvpram@gmail.com (P. Pavadai).

<https://doi.org/10.1016/j.ijbiomac.2026.154405>

Received 31 December 2025; Received in revised form 7 September 2026; Accepted 7 September 2026

Available online 10 September 2026

0141-8130/© 2026 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

PDK1 should therefore be considered a biologically important but clinically unvalidated therapeutic target.

This review critically examines the biology and therapeutic evidence for PDK1, with particular emphasis on its structural regulation, substrate-specific signaling, and cancer-associated functions. In addition to AKT, PDK1 phosphorylates several other non-AKT AGC-family kinases, including SGK1/3, RSK, S6K, and PKC isoforms [5]. PDK1 also plays a role in substrate docking via its PIF pocket and PH domain, and reports suggest that it may assemble specific protein complexes with PLC γ 1, 14-3-3 proteins, and other regulatory partners [6]. These non-catalytic observations are context-dependent and should not be taken as definitive explanations for clinical inhibitor failure. ATP-competitive PDK1 inhibitors can, in principle, inhibit activation-loop phosphorylation of multiple substrates, including AKT, SGK, S6K, and RSK. The degree and longevity of inhibition, however, are influenced by compound exposure, substrate priming, expression, localization, pathway feedback, and tumor context. PIF-pocket ligands, dual-site inhibitors, interface-disrupting agents, and PDK1-directed protein-degradation strategies are thus investigational strategies, not clinically validated alternatives [7,8].

The development of PDK1 inhibitors for oncological treatment has progressed slowly. The straightforward biomarkers for PDK1 activity or inhibition have not yet demonstrated utility [9]. Some exploratory aspects that might be of interest in the study of PDK1 in cancer include measurement of PDK1 copy number/expression, PTEN status, activating PIK3CA alterations, and pharmacodynamic phosphoprotein markers. AKT Thr308 is a direct PDK1-regulated activation-loop site, whereas NDRG1 Thr346 primarily reflects downstream SGK activity, and 4E-BP1 phosphorylation reflects mTORC1-associated signaling. For S6K1, Thr229 is the direct PDK1-regulated activation-loop site, whereas Thr389 is an mTORC1-regulated hydrophobic-motif priming site [10]. Phospho-PDK1 Ser241 is not a dynamic indicator of moment-to-moment upstream PI3K activity, as Ser241 is generally highly and relatively stable auto-phosphorylated. None of these proposed combinations of biomarkers have been validated in prospective clinical trials for patient selection with selective PDK1 inhibitors [11,12].

The aim of this review is to critically evaluate and organize the current evidence on the structure, substrate regulation, cancer-associated functions, therapeutic targeting, resistance mechanisms, and biomarker development of PDK1. We use a three-component framework to organize the evidence: (i) PIP3-dependent recruitment and activation of AKT; (ii) PIF-pocket-dependent regulation of non-AKT AGC kinases; and (iii) reported non-catalytic or scaffolding-associated functions of PDK1. This framework is a conceptual approach for comparing findings across studies and is not intended to be an experimentally validated causal model of inhibitor failure, treatment resistance, biomarker selection, or therapeutic response. Throughout the review, established biochemical mechanisms, results from individual experimental models, cross-tumor generalizations, and authors' hypotheses are therefore distinguished. Special focus is placed on substrate-specific regulation, adaptive signaling, PIF-pocket interactions, reported PLC γ 1- and 14-3-3-associated functions, and the constraints on the use of phospho-PDK1 Ser241 as a dynamic biomarker.

1.1. Search strategy and evidence evaluation

A systematic literature review was conducted to gather relevant literature on PDK1 signaling in cancer in accordance with the PRISMA Guidelines. The main keywords used were: PDK1, PDK1, 3-phosphoinositide-dependent protein kinase-1, PDK1 cancer, PI3K/AKT/mTOR pathway, AGC kinases, AKT, SGK, S6K, RSK, PKC, PIF pocket, PH domain, PDK1 inhibitors, PROTACs, drug resistance, biomarkers, and cancer therapy. These terms were combined using Boolean logic (AND, OR). Data were searched in databases such as PubMed, Scopus, Web of Science, and Google Scholar. A search of studies published between 1997 and May 2026 identified 4200 records. After removal of 780 duplicates, 3420 records underwent title and abstract screening. Of these, 2400 were excluded as outside the oncology-focused scope, unrelated to PDK1/PDK1 signaling, or referring to the PI3K/AKT pathway only in general terms without specific reference to PDK1/PDK1. A further 570 records were excluded prior to full-text review as conference abstracts, editorials or commentaries, non-English publications, or reports for

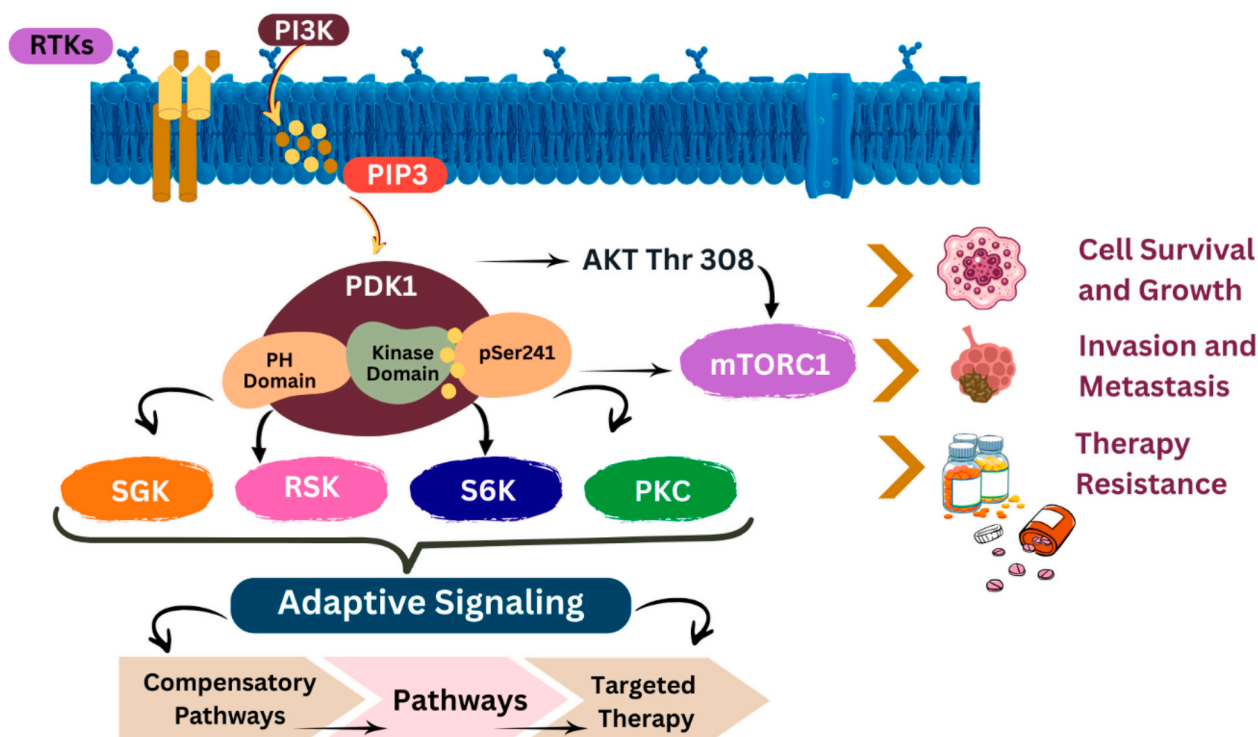


Fig. 1. PDK1-mediated PI3K signaling in cancer progression [13]

which the full text could not be retrieved. The remaining 450 full-text articles were assessed in detail for eligibility. Studies were retained if they addressed the structure, regulation, signaling functions, cancer-related roles, therapeutic targeting, biomarker relevance, or drug-resistance mechanisms of PDK1/PDPK1, and comprised original research articles, preclinical studies, clinical or patient-based investigations, or high-quality reviews relevant to the topic. Of these 450 articles, 205 were excluded at the full-text stage due to insufficient mechanistic detail, weak or indirect experimental evidence, or substantial overlap with findings already captured in other included studies. Finally, 245 studies met the eligibility criteria and were included in the review. The included studies were evaluated according to the type and strength of evidence. Biochemical studies, cell-based experiments, genetic studies involving siRNA, shRNA, or CRISPR approaches, animal studies, and clinical or patient-based studies were considered separately.

Greater emphasis was placed on findings supported by genetic, *in vivo*, or clinical evidence, while speculative mechanisms and future directions were presented cautiously. Computational prediction, such as molecular docking or molecular-dynamics simulation, is not definitive L1 evidence. Cellular pathway modulation is considered L2 evidence, and genetic dependency, animal efficacy, and human studies are considered separately as L3, L4, and L5 evidence.

1.2. Discovery of PDK1

Early 1990s research, especially on insulin signaling, revealed that phosphoinositide 3-kinase (PI3K) is rapidly activated by insulin receptor activation, resulting in the synthesis of phosphatidylinositol 3,4,5-trisphosphate (PIP3) at the plasma membrane [14]. At the same time, it was discovered that AKT/Protein Kinase B (PKB) is an essential regulator of cell growth, survival, and glucose metabolism. However, phosphorylation at Serine 473 (Ser473) in the hydrophobic motif and Threonine 308 (Thr308) in the activation loop was necessary for complete AKT activation [15]. Although it was established that AKT is recruited to the membrane through its Pleckstrin Homology (PH) domain, it was still unclear which particular kinase phosphorylates Thr308. This knowledge gap was addressed in 1997 with the isolation and cloning of 3-phosphoinositide-dependent protein kinase-1 (PDK1), the protein encoded by the PDPK1 gene [16]. Canonical human PDK1 is a 556-amino-acid serine/threonine kinase containing an N-terminal catalytic domain and a C-terminal phosphoinositide-binding pleckstrin-homology domain. Crucially, it was demonstrated that PDK1 phosphorylates AKT at Thr308 in a PIP3-dependent manner, providing the necessary molecular connection between AKT signaling and PI3K activation [17]. The identification of PDK1 established the molecular link between PI3K-generated phosphoinositides and activation-loop phosphorylation of AKT, and subsequently revealed its broader role in the regulation of AGC-family kinases [18].

1.3. PDK1 - a key regulator of AGC kinases

PDK1 is a central regulator of the AGC kinase family and can phosphorylate the activation loops of several kinases, including AKT, S6K, SGK, RSK, and PKC isoforms [19]. The phosphorylation of Ser241 in the PDK1 activation loop is essential for catalytic competence and is typically high and relatively constant at basal levels. Thus, the acute regulation of PDK1 signaling is primarily controlled by substrate recruitment, localization, substrate priming, and conformational interactions, but not by rapid switching of Ser241 phosphorylation [20]. In AKT, the PH domain of PDK1 and AKT binds to PIP3 generated by PI3K activation, which allows phosphorylation of AKT at Thr308. For several non-AKT AGC kinases, substrate recognition additionally involves docking of a phosphorylated hydrophobic motif into the PIF pocket of PDK1. The requirements for substrate priming differ among kinases and can involve mTORC1, mTORC2, ERK, lipid cofactors, expression level, and subcellular localization. Thus, PIF-pocket-

dependent signaling cannot be considered constitutive or generally independent of upstream PI3K-associated regulation [21]. This review covers the structure, regulation, substrate recognition, cancer-related roles, and new therapeutic strategies for PDK1. Investigational strategies such as allosteric modulators, PIF-pocket ligands, dual-site inhibitors, and protein-degradation strategies are discussed, with evidence largely from biochemical and preclinical studies.

2. PDK1: structural and functional overview

2.1. Gene, expression, and isoforms

The discovery of 3-phosphoinositide-dependent protein kinase-1 in rabbit skeletal muscle in 1997 marked a significant turning point in understanding AGC kinase regulation [22]. The human PDPK1 gene (Gene ID: 5170), which encodes the 3-phosphoinositide-dependent protein kinase-1 protein (PDK1), the canonical protein isoform (UniProt O15530-1; RefSeq NP_002604.1) and isoform 2 (UniProt O15530-2), was subsequently identified and characterized in human cells. Earlier literature used alternative gene names, including PDPK2, PDPK2P, and PRO0461; however, PDPK1 is the currently accepted gene symbol. To maintain nomenclature consistency throughout this review, PDPK1 is used exclusively to refer to the gene, whereas PDK1 refers to the encoded 3-phosphoinositide-dependent protein kinase-1 protein. The unrelated mitochondrial pyruvate dehydrogenase kinase 1 is referred to by its full name to avoid ambiguity. PDPK1 gene, located on chromosome 16p13.3, has 17 exons and encodes a protein of approximately 65 kDa, making PDK1 an essential component of intracellular signaling cascades [15]. PDK1 is broadly distributed in mammalian tissues, particularly in metabolically active organs such as the brain, liver, pancreas, heart, and skeletal muscle, underscoring its role in growth regulation and energy metabolism. The kidney, lung, and placenta exhibit moderate to low levels [23]. PDK1 is mostly cytoplasmic, but upon PI3K activation, it translocates to the plasma membrane. Notably, PDK1 is often overexpressed in breast, prostate, pancreatic, ovarian, and lung cancers.

Alternative splicing of the PDPK1 gene produces at least two transcript variants that generate different protein isoforms. The canonical Isoform A is a 556-amino-acid protein with an approximate molecular mass of 63–65 kDa [24]. It contains both the conserved N-terminal catalytic kinase domain and the C-terminal PH domain, which binds phosphoinositides and facilitates membrane recruitment. Isoform A is the most extensively studied isoform in signaling and disease-related investigations [25]. An alternative splice site in the 3' coding region gives rise to isoform B, which has a different C-terminus and lacks a portion of the PH domain. Its altered C-terminal region may influence subcellular localization, stability, phosphoinositide binding, and interactions with regulatory partners; however, its functional properties remain less extensively characterized than those of the canonical isoform [15]. Overall, these isoforms enhance PDK1's functional diversity by providing subtle regulatory variations while maintaining its central role as a kinase in PI3K/AGC signaling pathways.

2.2. Protein structure and functional domains

The unique structure of phosphoinositide-dependent protein kinase-1 (PDK1) allows it to phosphorylate a variety of substrates [26] such as AKT/PKB, S6 kinase (S6K), serum- and glucocorticoid-induced kinase (SGK), ribosomal S6 kinase (RSK), and numerous PKC isoforms. The combined action of PDK1's kinase domain, PH domain, and PDK1-interacting fragment (PIF) pocket, which together enable substrate binding and activation, provides protein flexibility [27].

2.2.1. Kinase domain

Human PDPK1 encodes 3-phosphoinositide-dependent protein kinase-1 (PDK1), which consists of 556 amino acids and contains the N-

terminal kinase domain and C-terminal pleckstrin homology (PH) domain. Although alternative splice isoforms have been annotated, the canonical isoform represents the primary reference sequence used in biochemical, structural, and pharmacological studies. The enzymatic core of PDK1 is the N-terminal kinase catalytic domain (residues 51–359) [28]. It adopts the conserved bilobal protein kinase fold, comprising a smaller β -sheet-rich N-lobe and a larger, predominantly α -helical C-lobe separated by the ATP-binding cleft. The activation loop contains Ser241, whose autophosphorylation is required for catalytic competence [29]. Ser241 phosphorylation is generally high and relatively stable under basal conditions, and mutation of this residue markedly reduces PDK1 activity as shown in Fig. 2. The smaller lobe of the domain also contains a hydrophobic docking groove called the PIF pocket [30]. The PIF pocket provides a docking surface for phosphorylated hydrophobic motifs present in several AGC-kinase substrates and contributes to substrate recognition and catalytic organization.

2.2.2. Pleckstrin Homology (PH) domain

The C-terminal PH domain of PDK1 is responsible for the binding of phosphoinositides, especially PIP3 and phosphatidylinositol-3,4-bisphosphate. Upon activation of class I PI3K, these lipids localize to the plasma membrane and bring PDK1 and PH-domain-containing substrates like AKT into close proximity. This membrane colocalization enables phosphorylation of AKT at Thr308. The PH domain also plays a role in conformational organization, subcellular localization, and interactions with regulatory partners. However, the binding of PIP3 should not be considered mainly as an on/off switch for Ser241 phosphorylation. The basal catalytic competence is provided by Ser241, which is usually in a highly auto-phosphorylated state, while the spatial availability of PDK1 to appropriate substrates is mainly regulated by phosphoinositide binding [31]. Therefore, the PH domain is more of a localization and regulatory module that is dependent on phosphoinositides than a simple on–off switch.

2.2.3. The PIF pocket

One distinctive characteristic of PDK1 is the PIF pocket, which is found in the kinase domain's N-terminal region [24]. It interacts with hydrophobic patterns on AGC kinase substrates, including S6K1, SGK1, RSK, and PRK2, by acting as a hydrophobic docking site. For several substrates, prior phosphorylation of the hydrophobic motif by an upstream kinase promotes docking into the PIF pocket. The priming kinase and regulatory requirements differ among substrates; examples include

mTORC1, mTORC2, and ERK-dependent mechanisms. Binding of a phosphorylated hydrophobic motif to the PIF pocket can stabilize catalytically favorable PDK1 conformations and enhance phosphorylation of selected substrates [32]. Mutagenesis research shows that phosphorylation of PIF-dependent substrates is specifically disrupted when important residues in the PIF pocket are changed. By contrast, AKT relies predominantly on PH-domain-mediated membrane colocalization and is therefore less dependent on PIF-pocket docking than substrates such as S6K, SGK, and RSK [33].

2.2.4. Substrate docking mechanisms

PDK1 uses complementary substrate-recognition mechanisms that vary between AGC-family kinases. In AKT, the PH domain of PDK1 and AKT bind to PIP3, which is generated by PI3K, and promote the phosphorylation of AKT at Thr308 [34]. For substrates such as S6K, SGK, RSK, and some PKC isoforms, recognition often requires docking of a phosphorylated hydrophobic motif into the PIF pocket of PDK1. These substrates also require specific priming events, expression levels, localization, lipid cofactors, and upstream regulators. This diversity allows PDK1 to combine multiple signaling inputs, but does not mean that all PIF-pocket-dependent substrates are always active or that they are always independent of PI3K-associated signaling [10,35].

2.3. Regulation of PDK1 activity

PDK1 activity and signaling output are regulated through a combination of basal activation-loop phosphorylation, phosphoinositide-dependent localization, substrate priming, PIF-pocket docking, protein interactions, and subcellular distribution [30]. The phosphorylation of Ser241 is essential for catalytic competence and is typically relatively stable, so acute regulation of the pathway is primarily achieved through substrate availability and context-dependent regulatory interactions.

2.3.1. Ser241 autophosphorylation and catalytic competence

The catalytic competence of PDK1 requires autophosphorylation of Ser241 in the activation loop. The biochemical and cellular evidence available suggests that Ser241 phosphorylation is generally high and relatively stable under basal conditions, and is not a rapidly inducible readout of upstream PI3K activation [34].

The Ser241 mutation significantly decreases catalytic activity, highlighting its essential role in maintaining the kinase's active conformation. Beyond the classical model in which phosphoinositide binding

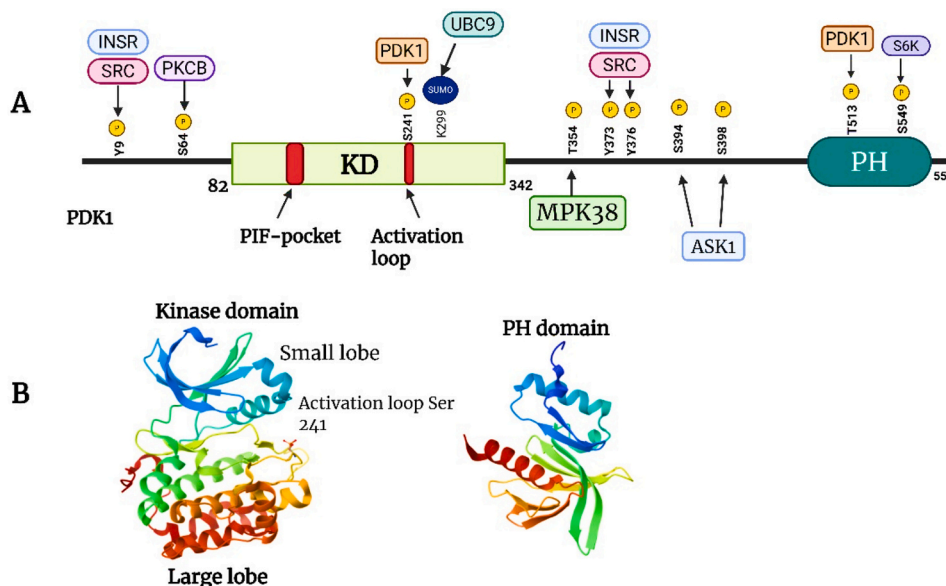


Fig. 2. (A) Primary structure of PDK1; (B) 3D structures of the kinase domain (PDB ID:4rrv) and the PH domain (PDB ID: 1w1g) [13]

primarily facilitates PDK1 membrane recruitment through the PH domain, recent structural and biophysical studies have proposed additional phosphoinositide-dependent regulatory mechanisms. These studies suggest that PIP3 or PI(3,4)P2 binding to the PH domain may relieve autoinhibitory kinase-PH domain interactions, enhance membrane-associated PDK1 organization, and promote conformational states that favor kinase activation and Ser241 autophosphorylation through mechanisms involving kinase-domain organization, including α G helix-mediated interactions [30]. A related structural analysis of the autoinhibited, linker-engaged conformation of PDK1 has further characterized how interdomain interactions may restrict activation-loop accessibility and regulate catalytic competency. However, these mechanisms have been primarily demonstrated using reconstituted biochemical and structural approaches and have not yet been established as the dominant regulatory mechanism of PDK1 activation in intact cells or tumors. Therefore, they should be considered an additional and complementary layer of phosphoinositide-dependent regulation rather than a replacement for the classical substrate-colocalization model described above. Importantly, these findings do not indicate that growth-factor-induced PIP3 production universally functions as a simple binary switch that converts Ser241 from an unphosphorylated to phosphorylated state in physiological cellular contexts [36].

PIP3 binding to the PH domain primarily regulates membrane localization, conformational organization, and colocalization with PH-domain-containing substrates such as AKT. In addition, PDK1 can phosphorylate a number of substrates in a PIF-pocket-dependent manner, without requiring co-recruitment to membranes by PIP3 [10]. However, these pathways are still substrate-specific and require substrate-specific priming, expression, localization, and regulatory inputs from mTORC1, mTORC2, ERK, and lipid cofactors [37]. Phospho-AKT Thr308 is a direct PDK1-regulated activation-loop readout, and S6K1 Thr229 similarly represents direct PDK1-mediated activation-loop phosphorylation [27]. In contrast, phospho-NDRG1 Thr346 primarily reflects SGK activity, S6K1 Thr389 reflects mTORC1-dependent hydrophobic-motif priming, and phospho-4E-BP1 primarily reflects mTORC1-associated signaling. These downstream pathway markers may indicate broader pathway modulation but should not individually be interpreted as direct evidence of PDK1 target engagement [38].

3. Biological functions of PDK1

PDK1 regulates a wide network of AGC-family kinases and has been linked to some protein-interaction and scaffolding activities. To bring these diverse findings together, we propose a three-component framework: (i) PIP3-dependent colocalization of PDK1 and AKT; (ii) PIF-pocket-dependent recognition of non-AKT AGC-kinase substrates; and (iii) reported non-catalytic or scaffolding-associated functions of PDK1. This framework is not meant to be a causal model of inhibitor failure, adaptive resistance, biomarker performance, or therapeutic response established in an experiment. The components are not mutually exclusive and can overlap depending on the substrate type, cellular localization, tumor type, pathway feedback, and treatment context [39].

3.1. Component 1: PIP3-dependent PDK1-AKT signaling

Receptor tyrosine kinases or G-protein coupled receptors are activated by growth factors, cytokines, and hormones, which can stimulate class I PI3K and promote the conversion of PIP2 to PIP3 at the inner leaflet of the plasma membrane. PIP3 is a membrane-docking site for PH domain-containing proteins such as PDK1 and AKT. They colocalize to promote the phosphorylation of AKT at Thr308 by PDK1 [40]. The strength and duration of this signaling depend on upstream receptor activation, PI3K activation, phosphatase activity (e.g., PTEN), receptor membrane localization, and pathway feedback. In certain tumor contexts, loss of PTEN or activating mutations of the components of the PI3K

pathway can lead to elevated levels of PIP3 and boost PDK1-AKT signaling [41].

3.1.1. PDK1-mediated activation of AKT

After co-localization at the membrane, PDK1 can phosphorylate AKT at Thr308 in the activation loop, a site required for AKT activation. Full activation also depends on phosphorylation of Ser473 in the hydrophobic motif by mTORC2, which promotes the active conformation of AKT, facilitating substrate recognition [42]. Phosphorylation of AKT at Ser473 is mediated predominantly by mTORC2 in most cellular contexts. The historical term “PDK2” referred to the activity responsible for Ser473 phosphorylation and should not be interpreted as a separate PDK1-related kinase [43]. Activated AKT phosphorylates multiple substrates involved in cell survival, metabolism, and proliferation. These effects include inhibition of pro-apoptotic regulators such as BAD, modulation of FOXO transcription factors, inhibition of GSK-3 β , stimulation of glucose uptake, and promotion of protein synthesis and metabolic adaptation [44].

3.1.2. Therapeutic relevance of PDK1-AKT signaling

ATP-competitive PDK1 inhibitors, such as GSK2334470, inhibit PDK1 catalytic activity and reduce activation-loop phosphorylation of multiple substrates, including AKT, SGK, S6K, and RSK. Their activity is therefore not intrinsically restricted to AKT or to Component 1. The magnitude of substrate inhibition depends on compound concentration, intracellular exposure, substrate priming, localization, expression, and treatment duration [45]. Inhibition of AKT Thr308 phosphorylation may reduce selected AKT-dependent outputs, but residual pathway activity can persist through mTORC2-associated regulation, non-AKT AGC kinases, pathway feedback, or parallel signaling networks. These findings provide possible mechanistic explanations for incomplete pathway suppression in particular experimental models, but they do not establish why selective PDK1 inhibitors have not progressed clinically [46].

3.2. Component 2: PIF-pocket-dependent regulation of non-AKT AGC kinases

In addition to AKT, PDK1 phosphorylates several AGC-family kinases whose recognition commonly involves docking of a phosphorylated hydrophobic motif into the PIF pocket. These substrates include S6K, SGK, RSK, and selected PKC isoforms. Unlike AKT, many of these substrates do not require shared PIP3-mediated membrane colocalization with PDK1. However, their activation is not constitutive or universally independent of PI3K-associated signaling. It depends on substrate-specific priming, abundance, localization, lipid cofactors, and regulatory inputs from pathways such as mTORC1, mTORC2, and ERK [27].

3.2.1. PIF-pocket-dependent substrate activation

The PIF pocket is a hydrophobic pocket in the N-terminal lobe of the kinase domain that recognizes phosphorylated hydrophobic motifs on substrate kinases [5]. This pocket is not a phosphorylation site, but rather a docking site for the phosphorylated hydrophobic motifs of the AGC kinases, which are then phosphorylated by PDK1 on their activation loops. Unlike AKT, many PIF-pocket-dependent substrates do not need to be co-localized on membranes to be activated by PIF. Their activation, however, is not constitutive but requires substrate-specific priming, expression, and localization, as well as inputs such as mTORC1/mTORC2 and ERK signaling. These substrates may therefore be less responsive to upstream PI3K inhibition, but are not universally unresponsive [30,47]. Some of the most important PIF-pocket-dependent substrates and their oncogenic role are presented in Table 1. S6K1 is primed through mTORC1-mediated phosphorylation of its hydrophobic motif at Thr389, after which PDK1 phosphorylates the activation loop at Thr229. This sequential regulation links nutrient and growth-factor signaling to ribosomal biogenesis and translation [48]. SGK isoforms are regulated through mTORC2-associated hydrophobic-

Table 1
Downstream targets regulated by PDK1.

Target kinase	Phosphorylation site(s)	Mechanism of activation	Cellular processes regulated	Evidence from cancer studies	References
AKT (PKB)	Thr308 (activation loop) - direct PDK1 target; Ser473 (hydrophobic motif) - mTORC2-mediated, context-dependent	PIP3-dependent membrane co-localization; PDK1 phosphorylates Thr308; Ser473 phosphorylation by mTORC2 contributes to maximal or substrate-selective AKT activity.	Cell survival, growth, proliferation, glucose metabolism, protein synthesis, apoptosis inhibition, metabolic regulation	Thr308 inhibition by GSK2334470 reduces tumor growth in multiple models; Ser473 effects vary by cell type and context	[61,62]
p70S6K (S6K)	mTORC1-mediated phosphorylation of the S6K1 hydrophobic motif at Thr389 promotes PIF-pocket-dependent docking, followed by direct PDK1-mediated phosphorylation of the activation loop at Thr229	PIF pocket-mediated docking requires mTORC1-mediated priming phosphorylation	Protein synthesis, ribosomal biogenesis, cell growth, cell size regulation, translation initiation	Triple targeting with AKT and RSK shows therapeutic benefit in B-cell lymphomas; compensatory activation in HNSCC	[63,64]
p90RSK (RSK)	Ser221 (activation loop) - direct PDK1 target	PIF pocket-mediated docking requires ERK-mediated priming	Translation regulation, cell proliferation, survival, MAPK pathway effector	Inhibited by GSK2334470; RSK2 is a pivotal downstream effector in B-cell lymphomas	[65]
SGK (SGK1/2/3)	Activation loop Thr (site varies by isoform: SGK1 Thr256)	PIF-pocket-dependent docking following isoform- and context-specific priming; regulation may involve mTORC2 and phosphoinositide-associated localization.	Ion transport, cell survival, growth, sodium channel regulation, epithelial transport	Regulated by PDK1 in multiple cancer types; contributes to AGC kinase network redundancy	[23,66]
PKC isoforms (PKC α , PKC β , PKC δ , PKC ϵ , PKC ζ , PKC ι/λ)	Activation loop Thr (varies by isoform)	PIF pocket-mediated docking; lipid cofactor-dependent	Migration, cytoskeletal dynamics, signal transduction, invasion, metastasis, and cell polarity	PKC α activation in HNSCC; PKC regulation in breast cancer migration; PKC η phosphorylates PDK1	[63,67]
PRK1/2 (PKN1/2)	Activation loop Thr (PRK2 Thr958)	PIF pocket-mediated; regulated by Rho GTPases; Z/TM site phosphorylation modulates PDK1 interaction	Cytoskeletal organization, cell migration, Rho signaling	Specific regulatory mechanisms for PRK-PDK1 interaction are distinct from those of other AGC kinases	[68]
mTOR (indirect)	Ser2448 and Ser2481 (observed changes, not direct PDK1 targets)	Indirect regulation through AKT and other intermediates; feedback loops	Autophagy, protein synthesis, cell growth, nutrient sensing, metabolic regulation	mTOR phosphorylation reduced by PDK1 inhibitor combinations in MM; mTORC1 activity decreased in RCC	[61,62]
PLC γ 1 (non-canonical)	Tyr783 (observed in PDK1-dependent manner)	PDK1/PLC γ 1 complex formation; PH domain interaction	Calcium signaling, cell migration, invasion, lipid signaling	PDK1-PLC γ 1 interaction critical for breast and melanoma invasion; disrupted by 2-O-Bn-InsP5	[69]
PTEN (regulatory interaction)	Ser380, Thr382/383 (stability regulation)	PDK1 inhibition affects PTEN phosphorylation and localization	Tumor suppressor function, PI3K pathway negative regulation, protein stability	PDK1 inhibition increases nuclear PTEN accumulation in MM; modulates PTEN degradation	[62]

motif priming followed by activation-loop phosphorylation by PDK1. In selected experimental models, SGK1 or SGK3 can phosphorylate TSC2 and contribute to maintenance of mTORC1 signaling when AKT activity is reduced [49]. The protein RSK links the MAPK and PI3K pathways by acting as an ERK-mediated priming step prior to activation loop phosphorylation by PDK1 and by increasing PDK1's oncogenic functions, including transcriptional regulation and MAPK-dependent tumor environments. The regulation of PKC isoforms through PIF-pocket engagement is lipid cofactor-dependent or independent and is associated with cell polarity, cytoskeletal dynamics, adhesion turnover, and invasion. These functions suggest a potential link between PDK1-regulated PKC signaling and cytoskeletal or invasive phenotypes, although the contribution varies among PKC isoforms and tumor contexts [50].

3.2.2. Context-dependent SGK-mTORC1 bypass signaling in therapeutic resistance

A context-dependent adaptive mechanism is the maintenance of mTORC1 signaling in the presence of low AKT activity via SGK1 or SGK3. In some models, SGK can phosphorylate TSC2, thereby reducing the inhibitory effect of the TSC1–TSC2 complex on Rheb and promoting phosphorylation of S6K and 4E-BP1 by mTORC1 [51]. This mechanism has been demonstrated in certain experimental systems and is not necessarily the primary resistance pathway across all tumor types. In

selected breast cancer models, prolonged PI3K α inhibition has been associated with increased PDK1–SGK1/3 signaling and the persistence of mTORC1 activity. Catalytic inhibition of PDK1 with GSK2334470 or genetic suppression of PDK1 restored sensitivity to PI3K α inhibition in those models and reduced selected downstream mTORC1 readouts [9]. These findings support a model-specific role for PDK1–SGK signaling in adaptive resistance, but clinical relevance and generalizability across tumor types remain to be established

3.3. Component 3: reported non-catalytic and scaffolding-associated functions

Several studies have proposed that PDK1 contributes to signaling through protein–protein interactions, complex assembly, localization, or conformational regulation in addition to its catalytic activity [52]. Representative examples include interactions involving PLC γ 1, 14–3-3 proteins, Hsp90, and cytoskeletal-signaling components. The evidence for these functions is context-dependent and derives mainly from biochemical and cellular studies. It remains uncertain whether these activities are entirely independent of catalytic function or contribute to the limited antiproliferative effects of specific ATP-competitive inhibitors. Strategies designed to disrupt protein interfaces or degrade PDK1 may affect both catalytic and interaction-associated functions, but

this remains an investigational hypothesis rather than an established therapeutic advantage [7].

3.3.1. Adaptor regulation and conformational control

Reported studies suggest that 14-3-3 proteins can interact with phosphorylated PDK1 and influence its conformation, localization, and access to selected substrates [2]. Experimental stabilization or disruption of this interaction has been associated with altered phosphorylation of AKT and SGK, suggesting a possible regulatory role; however, the generality and tumor-specific relevance of this mechanism require further validation [53].

Altered adaptor expression or post-translational regulation may modify these interactions in cancer cells, but direct evidence linking specific oncogenic alterations to clinically relevant PDK1–14-3-3 dysregulation remains limited [54]. Another regulatory aspect concerns the Hsp90 chaperone axis: Hsp90 binds and stabilizes PDK1, blocking its degradation by the proteasome and thereby maintaining its catalytic activity and scaffolding conformation [55]. Hsp90 inhibition may therefore indirectly alter PDK1 abundance and signaling; however, because Hsp90 regulates numerous client proteins, these effects cannot be attributed specifically to disruption of PDK1-associated scaffolding functions.

3.3.2. PDK1-associated cytoskeletal and invasive signaling

PDK1 has been associated with Rho–ROCK-dependent cytoskeletal regulation in selected cellular models [56]. These studies suggest that PDK1-related protein interactions or signaling complexes may influence actomyosin contractility, focal-adhesion turnover, membrane dynamics, and directional migration. Such effects have been linked to downstream components including myosin light chain and LIM kinase/cofilin-associated pathways.

These observations provide a possible mechanism through which PDK1 may contribute to invasive phenotypes beyond canonical AKT signaling. However, the extent to which these effects are independent of PDK1 catalytic activity, broadly conserved across tumor types, or therapeutically targetable remains uncertain. Combined targeting of catalytic PDK1 activity and cytoskeletal pathways therefore represents a testable preclinical hypothesis rather than an established strategy for preventing metastasis [57].

3.4. Integration of the proposed three-component framework

The proposed framework highlights overlapping aspects of PDK1 biology rather than mutually exclusive signaling modes. Catalytic PDK1 inhibitors can, in principle, reduce activation-loop phosphorylation of AKT, SGK, S6K, and RSK. The extent of inhibition varies according to compound exposure, substrate priming, cellular localization, pathway feedback, and tumor context. PIF-pocket ligands may alter substrate docking, whereas interface-disrupting or protein-degradation approaches may additionally affect selected protein interactions. Evidence for these latter strategies remains predominantly biochemical and preclinical [58].

The framework does not establish the causes of unsuccessful clinical development, nor does it predict that a specific combination strategy will be effective. Instead, it identifies experimentally testable questions concerning substrate-specific inhibition, adaptive signaling, protein interactions, and tumor-context dependence. Combination strategies involving PI3K, AKT, mTOR, SGK, or other pathway components should therefore be evaluated individually based on direct target engagement, pharmacodynamic effects, genetic dependence, toxicity, and *in vivo* efficacy [59]. Pharmacodynamic assessment should distinguish direct PDK1-regulated phosphorylation events from downstream pathway markers. AKT Thr308 is directly regulated by PDK1, while S6K1 Thr229 represents the direct PDK1-regulated activation-loop site of S6K1 [51]. In contrast, NDRG1 Thr346 is primarily a downstream readout of SGK activity, S6K1 Thr389 reflects mTORC1-dependent priming, and 4E-BP1

phosphorylation reflects mTORC1-associated signaling. These downstream markers can provide complementary information on pathway suppression or adaptive signaling but do not independently demonstrate direct PDK1 target engagement [60].

Table 1 summarizes PDK1-mediated regulation of AKT and selected non-AKT AGC-family kinases, together with reported non-canonical interactions. The table highlights the diversity of PDK1-regulated substrates and associated processes, while acknowledging that the strength and context of evidence vary across targets.

4. PDK1 in cancer: expression, dysregulation, and biomarker associations

4.1. PDK1 expression in different cancers

Altered PDK1 copy number or transcription, and increased PDK1 protein expression, have been reported in several solid and hematological malignancies. However, the strength and nature of evidence vary substantially among tumor types. Some cancers have patient-cohort data linking PDK1 or PDK1 measurements to outcomes, whereas others are supported mainly by cell-based, genetic, or animal studies. PDK1 should therefore be considered a context-dependent signaling regulator rather than a uniformly dominant oncogenic driver. The principal findings, evidence type, and limitations across cancer types are summarized in Table S1.

4.1.1. Breast cancer

PDK1 copy-number gain and elevated PDK1 protein expression have been reported in subsets of breast cancer, frequently in association with alterations such as ERBB2 amplification, PIK3CA mutation, or PTEN loss [70]. Experimental mammary models suggest that increased PDK1 signaling can enhance HER2-associated tumor development, migration, and anchorage-independent growth, while PDK1 alone may be insufficient to initiate tumor formation (Fig. 3) [71]. In a retrospective cohort of 172 primary breast cancers, PDK1 amplification was associated with reduced overall survival; however, this finding requires independent prospective validation [72]. Independent validation used protein lysate arrays from 478 samples, corroborating elevated PDK1 protein expression in most tumors, with immunohistochemistry positivity observed in 72–88% of instances across studies [73]. Elevated PDK1 may reduce the efficacy of PI3K inhibitors, suggesting a role in drug resistance, and is correlated with poor patient survival [74]. Pharmacological and genetic studies provide preclinical rationale for evaluating PDK1 together with HER2- or PI3K-pathway-directed treatment, but clinical efficacy has not been established [75].

4.1.2. Lung cancer

Elevated PDK1 expression or signaling has been reported in non-small-cell lung cancer and has been associated with advanced disease, lymph-node metastasis, and resistance to EGFR-directed therapy in selected studies [77]. In osimertinib-resistant cellular models, increased phosphorylation of PDK1 at Ser241 and a marked shift in osimertinib sensitivity were reported, while genetic or pharmacological PDK1 suppression partially restored drug responsiveness. These results provide model-specific evidence that PDK1-associated signaling can contribute to adaptive resistance; they do not establish phospho-Ser241 as a validated predictive biomarker [78]. PDK1-related effects on metabolism, survival and invasion are likely mediated indirectly through AKT, SGK, PKC and mTOR-associated pathways rather than through direct metabolic-enzyme activity [79]. Detailed findings are summarized in Table S1.

4.1.3. Esophageal Squamous Cell Carcinoma (ESCC)

Increased PDK1 expression has been reported in esophageal squamous-cell carcinoma relative to adjacent tissue and was associated with poorer overall survival in a retrospective multivariable analysis

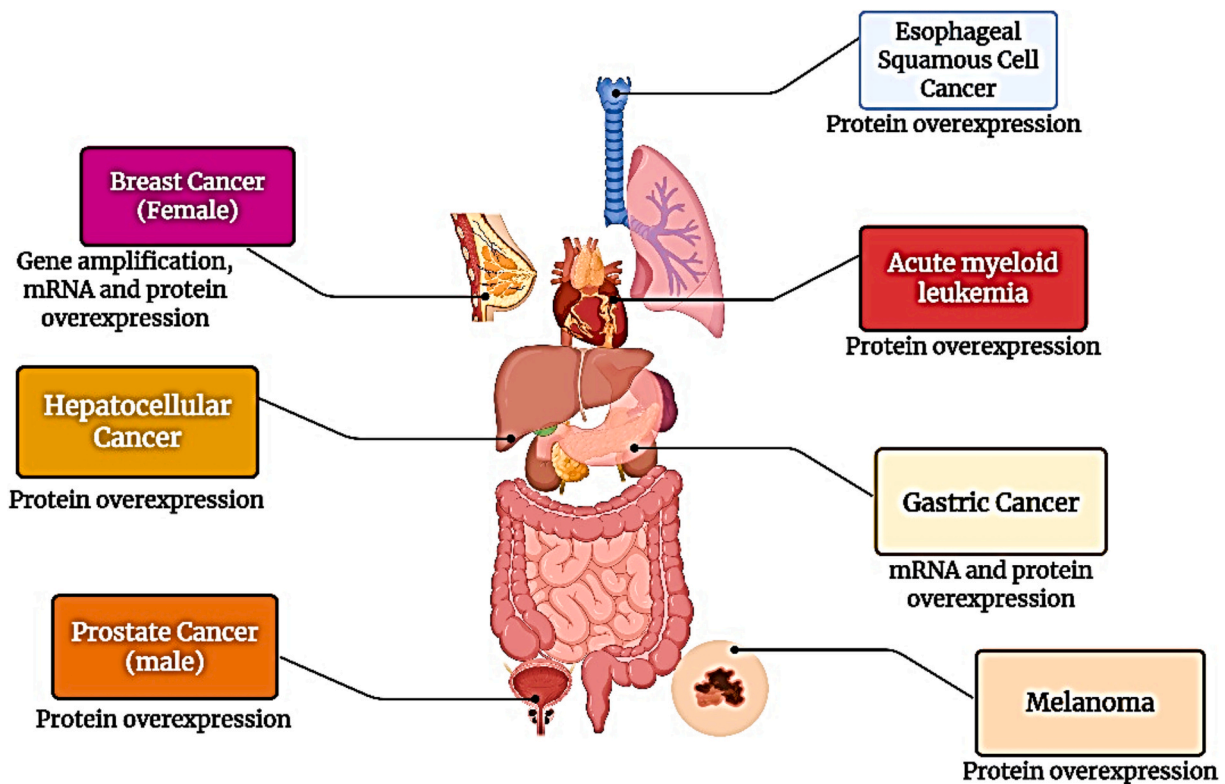


Fig. 3. Role of PDK1 in different cancers [76].

[80]. siRNA-mediated suppression of PDPK1 reduced motility and invasion in cultured ESCC cells, providing preclinical evidence of a functional contribution [81]. These findings require validation in independent patient cohorts and *in vivo* models. Detailed data are summarized in Table S1.

4.1.4. Gastric cancers

Elevated PDK1–AKT signaling has been associated with proliferation, survival, and invasion in gastric-cancer models. In a retrospective cohort of 156 patients, high PDK1 expression was associated with lower 5-year overall survival [82]. This finding supports a candidate prognostic association, but standardized detection methods and prospective external validation are lacking. Mechanistic and clinical findings are summarized in Table S1.

4.1.5. Hepatocellular Carcinoma (HCC)

PDK1-associated AKT–mTOR signaling has been linked to proliferation, angiogenesis, and metabolic adaptation in hepatocellular carcinoma models. In a retrospective cohort of patients undergoing curative resection, elevated PDK1 expression was associated with shorter post-operative overall survival [83]. Preclinical studies have also suggested that PDK1 suppression may alter radiosensitivity. However, neither PDK1 expression nor phospho-PDK1 Ser241 has been prospectively validated as a predictive marker in hepatocellular carcinoma. Detailed evidence is summarized in Table S1.

4.1.6. Acute Myeloid Leukemia (AML)

Increased PDK1 expression has been reported in a subset of acute myeloid leukemia samples and has been associated with PKC-mediated survival signaling. Genetic or pharmacological suppression of PDK1 reduced survival or colony formation in selected primary and cultured AML models, while effects on normal progenitor cells differed according to the experimental system [84]. These findings provide biochemical, cellular, and limited *ex vivo* evidence but do not establish PDK1 as a clinically validated dependency or predictive biomarker in AML [85].

4.1.7. Melanoma

In melanoma models, PDK1 signaling has been linked to survival, invasion, and resistance in genetic contexts involving BRAF or NRAS activation and PTEN loss [86]. Genetic suppression of PDPK1 delayed tumor growth or metastasis in selected preclinical models, supporting a context-dependent functional contribution [87]. However, clinical evidence linking PDK1 expression or phosphorylation at Ser241 to recurrence, treatment response, or overall survival remains limited and inconsistent [88]. PDK1 should therefore be regarded as an investigational signaling dependency in melanoma rather than a validated prognostic or predictive marker. [88]. Collectively, these studies indicate that the consequences of altered PDK1 signaling depend on tumor lineage, genomic background, substrate availability, and treatment context. In some models, PDK1 contributes predominantly to survival and proliferation; in others, its reported effects involve migration, invasion, metabolic adaptation, or drug resistance. Evidence should therefore be interpreted in light of the specific experimental model and should not be generalized across cancer types. Table S1 provides a comparative summary of the principal findings and their limitations.

4.2. PDK1 – mechanisms of dysregulation

4.2.1. Gene amplification

One way to increase PDK1 abundance in cancer is through genomic gain of the PDPK1 locus on chromosome 16p13.3. In breast cancer, it was reported that total PDK1 protein and PDPK1 mRNA expression were increased in most of the tumors compared to normal mammary tissue adjacent to the tumor. In the study by Maurer et al., 21% (27/129) of breast tumors had five or more copies of PDPK1, defined as increased copy-number gain. Tumors with higher PDPK1 copy number had higher PDPK1 transcript levels, suggesting that genomic gain contributes to higher PDK1 expression. Increased PDK1 protein levels were also seen in tumors lacking PDPK1 copy-number gain, however, suggesting that copy-number gain is just one of multiple mechanisms that control PDK1 expression and abundance [89].

In the analyzed cohort, increased PDPK1 copy number was associated with poor clinical features and was enriched in breast tumors with upstream alterations in the PI3K pathway, such as ERBB2 amplification, PTEN loss, and PIK3CA alterations [89]. In the reported models, PDK1 overexpression alone was insufficient to transform mammary epithelial cells, but it did increase signaling, growth, migration, and ERBB2-induced tumor formation in these cells, all of which are associated with PI3K/AKT signaling. These results suggest that the upregulation of PDK1 is a pathway amplifier that can work in concert with upstream oncogenic changes in the PI3K pathway, but is not a standalone transforming event. Phosphorylation of PDK1 at Ser241 should be analyzed independently of PDPK1 copy-number gain. The activation-loop auto-phosphorylation site Ser241 is essential for catalytic activity and is typically highly phosphorylated under basal conditions. In an independent study of breast cancer tissues, moderate-to-high phospho-PDK1 Ser241 staining was observed in 86% of high-grade/metastasized breast cancers, reflecting phospho-PDK1 Ser241 abundance and not correlating with the frequency of PDPK1 amplification reported by Maurer et al. [89]. The immunohistochemical staining for phospho-Ser241 may vary among tumors, potentially due to differences in PDK1 regulatory state, PDK1 abundance, cellular context, or alterations in signaling pathways. Thus, phospho-PDK1 Ser241 staining should not be used as a direct surrogate for PDPK1 copy number or overall PDK1 expression. The copy number of PDPK1, the total abundance of PDK1, and phospho-PDK1 Ser241 are related but distinct molecular measurements and should be interpreted separately when determining the status of the PDK1 pathway [89,90]. Copy-number gain of the 16p13.3 region, which includes PDPK1, has also been reported in prostate cancer, and higher gains have been observed in lymph-node metastases and castration-resistant disease than in primary tumors. These results suggest that PDPK1-induced genomic changes are not exclusive to breast cancer, but their prevalence and impact are context-dependent on the tumor type [91].

4.2.2. Epigenetic and post-transcriptional regulation

Several microRNAs, including miR-375, miR-503, miR-155-5p, miR-1271 and miR-409-3p, have been reported to regulate PDPK1 transcript abundance in selected cancer models [92]. Reduced expression of these microRNAs may therefore increase PDPK1 mRNA or PDK1 protein levels and enhance downstream signaling, although the relevance differs among tumor types. PDK1 stability may also be influenced by chaperone interactions involving Hsp90. Interactions with PLC γ 1, ROCK-associated

pathways, and adaptor proteins are better described as reported signaling or localization mechanisms rather than established causes of increased PDK1 abundance [93].

4.2.3. Feedback mechanisms

PDK1-regulated signaling is influenced by feedback within the PI3K–AKT–mTOR and broader AGC-kinase networks. Suppression of one pathway component can alter upstream receptor activity, substrate priming, or signaling through SGK, RSK, S6K, and PKC in a model-dependent manner [21]. In selected PIK3CA-mutant breast-cancer systems, SGK3-associated signaling has contributed to reduced dependence on AKT, as shown in Fig. 4. These observations support context-dependent adaptive signaling but should not be described as universal rerouting from AKT to SGK or RSK. Interactions between PDK1 and 14–3–3 proteins have also been reported to influence PDK1 conformation and substrate phosphorylation in biochemical and cellular systems [94]. The relevance of this mechanism to patient tumors and treatment resistance remains uncertain.

4.3. Functional biomarker basis

PDPK1 copy number and mRNA expression, total PDK1 protein, phospho-PDK1 Ser241, PTEN status, PIK3CA alterations, and downstream phosphoproteins have been evaluated as possible biomarkers of PDK1-associated signaling. However, none has been prospectively validated for selecting patients for selective PDK1-directed therapy [95].

Phosphorylation of PDK1 at Ser241 is required for catalytic competence but is generally high and relatively stable. Consequently, phospho-Ser241 should not be interpreted as a real-time measure of upstream PI3K activity. In tumor samples, elevated phospho-Ser241 may reflect increased total PDK1 abundance, as shown in Table 2 and the assay conditions, or altered cellular distribution. Dynamic pathway assessment should distinguish proximal PDK1-regulated substrate phosphorylation from downstream pathway output. AKT Thr308 and S6K1 Thr229 represent direct PDK1-regulated activation-loop sites, whereas NDRG1 Thr346 primarily reflects SGK activity, S6K1 Thr389 reflects mTORC1-dependent priming, and 4E-BP1 phosphorylation primarily reflects mTORC1-associated signaling, as shown in Fig. 5.

A composite biomarker strategy incorporating genomic, expression, and downstream signaling measurements may be useful for future investigation. However, proposed panels remain exploratory and require standardized assays, predefined thresholds, independent

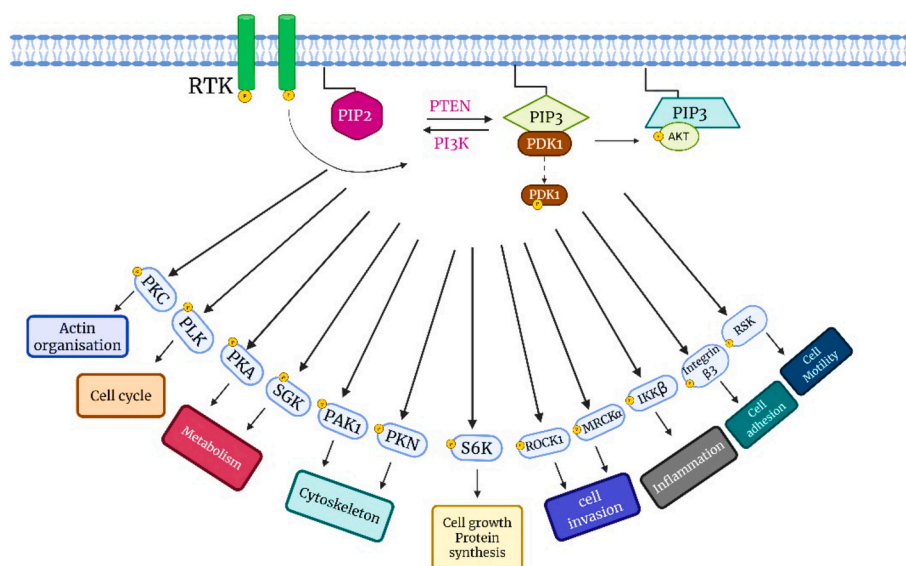


Fig. 4. PDK1-mediated downstream substrates and effectors [13].

Table 2
Biomarker evidence in different cancers.

Cancer type	Evidence/biomarker correlation	Findings	References
Breast Cancer	Amplification of the PDK1 gene and higher levels of phospho-PDK1 (Ser241) are associated with a worse prognosis, increased metastasis, and tamoxifen resistance.	Prognostic biomarker indicating likely failure of endocrine therapy.	[96]
Ovarian Cancer	Strong expression is detected in malignant tumors but not in normal tissue. Higher PDK1 mRNA levels are associated with decreased overall survival and accelerated disease progression and recurrence.	Prognostic biomarker and indicator of chemoresistance	[97]
Hepatocellular Carcinoma	Higher PDK1 mRNA and protein levels are associated with a shorter time to recurrence (TTR) and lower overall survival. Overexpression occurs in approximately 45% of patients and is associated with poor prognosis and resistance, primarily because leukemia stem cells are more resilient.	Prognostic biomarker	[98]
Acute Myeloid Leukemia (AML)	Regular amplification and overexpression are associated with tumor grade, invasion, metastasis, and recurrence.	Prognostic and predictive biomarker.	[99]
Prostate, Gastric, Lung, and Melanoma		Diagnostic and prognostic marker.	[87]

validation, and prospective testing. Current evidence supports candidate prognostic associations in selected retrospective cohorts, but does not establish PDK1 as a validated diagnostic, predictive, or treatment-selection biomarker [9].

In addition, PDK1 contributes to immune cell signaling and metabolism. As summarized in Table 2, available biomarker data associating PDK1 expression or activation with prognosis or treatment response are presented. The available evidence therefore supports the evaluation of PDK1-associated biomarkers together with mechanistically defined pathway readouts, including p-AKT Thr308, p-S6K1 Thr229, SGK-associated p-NDRG1 Thr346, mTORC1-associated p-S6K1 Thr389 and p-4E-BP1, as well as PTEN and other molecular context markers.

Future studies should evaluate PDK1 copy number or expression together with total PDK1 protein, phospho-Ser241, and dynamic downstream phosphoproteins using standardized assays and predefined analytical thresholds. Because phospho-Ser241 is relatively stable, it should be interpreted mainly as an exploratory measure of PDK1 abundance or catalytic competence rather than real-time PI3K-pathway activity. Prospective studies must determine whether any composite profile provides independent prognostic or predictive value beyond established tumor-specific biomarkers [9].

5. Preclinical evidence for PDK1 dependency and therapeutic targeting

5.1. Genetic suppression and functional-dependency studies

Genetic suppression of PDK1 by siRNA, shRNA, or CRISPR/Cas9 gives L3 evidence for a functional role of PDK1 in specific cancer models. These strategies can be used to differentiate PDK1 phenotypes from the side effects of drug inhibitors. The power of genetic evidence is dependent on the use of independent targeting sequences, confirmation of protein depletion, exclusion of clonal adaptation, and, if possible, rescue by re-expression of wild-type or functionally modified PDK1. Malignant pheochromocytoma cells treated with siRNA against PDK1 showed decreased cell proliferation and motility, increased cell death, and decreased AKT Thr308 phosphorylation, with relatively small changes at Ser473 [100]. In ovarian-cancer models, PDK1 suppression was

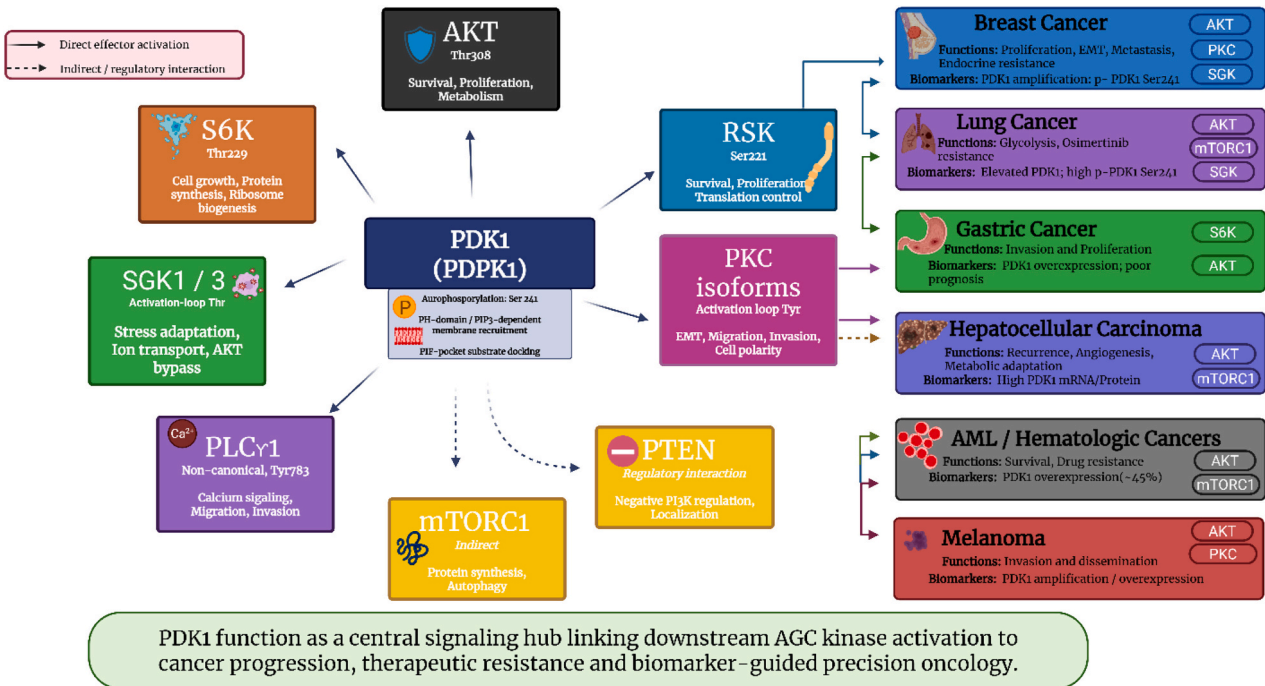


Fig. 5. PDK1 as a central signaling hub in cancer progression and therapeutic resistance [2].

correlated with decreased adhesion, migration, invasion and angiogenic phenotypes, as well as decreased $\alpha 5\beta 1$ -integrin expression. Stable shRNA-mediated suppression in selected breast cancer models also reduced proliferation, migration, and tumor growth [101]. These results provide model-specific L3 genetic evidence for PDK1's contribution to selected proliferative, survival, and invasive phenotypes. If genetically modified cells have been tested in xenograft or other animal models, the results of the tumor growth tests also give L4 evidence. However, results from individual cancer models should not be generalized to imply that all tumors depend on PDK1 [13].

5.1.1. CRISPR/Cas9 knockout (KO) and functional genomics

CRISPR/Cas9 studies are more convincing for L3 evidence if the phenotype is repeated in independent clones and reversed by re-expression of the target, as shown in Table S2. In NRAS-mutant melanoma models, PDK1 deletion enhanced sensitivity to MEK inhibition and was linked to decreased AKT- and PKC-mediated signaling and activation of gasdermin-E-mediated pyroptotic responses. In some melanoma models, deletion of PDK1 has also been shown to decrease clonogenic or tumor-forming capacity. The results suggest that PDK1 contributes to the survival and treatment response of melanoma cells in a context-dependent manner, but cannot be extrapolated to all melanoma subtypes [71].

In EGFR-mutant non-small-cell lung cancer models resistant to osimertinib, PDK1 knockout restored drug sensitivity and reduced selected PI3K-AKT-mTOR pathway readouts. Re-expression of the wild-type PDK1 reportedly restored the resistant phenotype, giving more on-target support than knockout alone. Where these effects have been reproduced in xenograft models, they offer L4 *in vivo* evidence in addition to L3 genetic evidence. However, the significance of this dependency for patients and other mechanisms of EGFR-inhibitor resistance is yet to be determined [9]. In summary, genetic studies have confirmed a functional role of PDK1 in tumor growth, survival, migration, or resistance to treatment in specific experimental systems. They do not show universal PDK1 dependency in cancer types. Moreover, simple knockdown or knockout is not sufficient to differentiate catalytic from non-catalytic functions unless coupled with rescue experiments with wild-type, kinase-inactive, or interaction-deficient PDK1 variants [87].

The studies in this section offer mostly L3 genetic-dependency evidence and L4 support in models involving genetically defined xenografts or animal experiments. Currently, there is no clinical evidence that PDK1 dependency can be used as a patient selection criterion. Genetic evidence also does not necessarily confirm the specificity of pharmacological compounds unless phenocopy, rescue, or inhibitor-resistant reconstitution experiments are conducted.

5.2. Therapeutic targeting of PDK1: mechanisms, challenges, and emerging strategies

PDK1 has emerged as a prospective therapeutic target due to its crucial role in regulating AGC kinases within the PI3K signaling pathway. Targeting PDK1 is challenging due to its role as a signaling hub that regulates numerous downstream substrates critical for cancer growth, metabolism, and survival [76]. A range of chemicals, encompassing both manufactured small molecules and natural compounds, has been reported to inhibit PDK1 signaling. Nevertheless, several studies classify medicines as PDK1 inhibitors primarily based on downstream outcomes, such as reduced AKT phosphorylation, without demonstrating direct target contact [13]. Careful distinction between direct catalytic inhibitors, multikinase compounds with PDK1 activity, PIF-pocket ligands, pH-domain-directed compounds, and indirect pathway modulators is required for pharmacological studies of PDK1. These compounds are not equally good at providing direct or selective evidence of PDK1 engagement. They may also have off-target kinases, parallel pathways, or non-PDK1 effects *in vivo* or in cells [102]. The L1-L5 framework is used throughout this section. L1 is direct

biochemical or target-engagement evidence; L2 is cellular pharmacological evidence; L3 is genetic dependency or target-specific rescue; L4 is *in vivo* evidence; and L5 is PDK1-specific clinical validation. Biochemical selectivity and target-specific validation are required to demonstrate direct PDK1 inhibition, which is not considered cellular or animal antitumor activity [103].

5.2.1. Small-molecule inhibitors and modulators of PDK1-associated signaling

Small molecules that interact with PDK1 signaling can be classified by their primary mechanism and selectivity. GSK2334470 is a relatively selective ATP-competitive PDK1 tool compound. BX-795 is a multi-kinase inhibitor with biochemical activity against PDK1 and TBK1, IKK ϵ , and other kinases. AR-12/OSU-03012 is a celecoxib-derived pleiotropic investigational compound with reported PDK1-associated activity. PHT-427 does not directly inhibit the ATP-binding site of PDK1, but rather blocks the recruitment of both PDK1 and AKT to the membrane via the PH domain. These compounds are not grouped together as a class of selective PDK1 inhibitors [104].

5.2.1.1. ATP-competitive inhibitors. ATP-competitive inhibitors bind to the catalytic ATP-binding site of PDK1 and may inhibit the activation-loop phosphorylation of several AGC-family substrates such as AKT, SGK, S6K, and RSK. The extent of inhibition varies with compound selectivity, intracellular exposure, substrate priming, localization, and experimental context. While direct catalytic inhibition is biochemical proof, conserved ATP-binding pockets throughout the kinome can make highly selective compounds difficult to develop.

GSK2334470 is a potent and relatively selective ATP-competitive PDK1 tool compound with reported low-nanomolar biochemical activity against the PDK1 catalytic domain, as shown in Table S3. Cellular studies have shown that activation-loop phosphorylation of SGK, S6K, and RSK is inhibited and AKT Thr308 phosphorylation is reduced in a context-dependent manner. The phosphorylation of AKT at Ser473 is not directly inhibited, as it is mainly regulated by mTORC2. GSK2334470 has been tested in several models of multiple myeloma, renal cancer, and breast cancer, and decreases in certain PDK1-dependent phosphoproteins, proliferation, or tumor growth have been observed. In general, however, rescue with an inhibitor-resistant PDK1 variant has not been performed for these compounds, and genetic suppression of PDK1 does not necessarily prove that all the pharmacological effects of GSK2334470 are on-target. Biochemical and preclinical activity of GSK2334470 has been published, but not the rationale for its lack of clinical development. Possible explanations include pharmacokinetic exposure, metabolic stability, therapeutic-window limitations, and development priorities, but none have been confirmed. GSK2334470 has strong L1 biochemical evidence and L2 cellular evidence, with L3 evidence from related genetic-dependency studies and L4 evidence in selected animal models. There is no clinical validation for PDK1 [45]. ATP-competitive inhibitors directly inhibit catalytic activity, but do not target protein-protein interfaces. The reported PDK1-associated scaffolding and interaction functions are not necessarily preserved in the catalytic inhibition state, nor is it clear if this is responsible for the limited antiproliferative activity in specific models [105].

BX-795 is biochemically active against PDK1, but also inhibits TBK1, IKK ϵ , NUA1, and other kinases at similar or higher potency [106]. Cellular studies have reported changes in AKT/S6K signaling, glycolysis, epithelial-to-mesenchymal-transition-associated markers, and sensitivity to chemotherapy or radiotherapy. These effects, however, cannot be specifically attributed to PDK1 without the use of selective comparator compounds, PDK1 genetic phenocopy, or target-specific rescue experiments. Therefore, BX-795 is not a selective PDK1 inhibitor, but rather a multikinase inhibitor with PDK1 activity. Use in TBK1-related applications, such as immune-cell or CAR-T manufacturing studies, does not constitute clinical validation of PDK1-directed therapy. BX-795

has non-selective L1 biochemical evidence and indirect L2 cellular evidence. There is no PDK1-specific L3, L4 or L5 evidence [107].

AR-12/OSU-03012 is a celecoxib-derived pleiotropic investigational compound that has been reported to affect PDK1-associated survival signaling. However, AR-12 also modulates multiple cellular processes, including endoplasmic reticulum stress, GRP78-associated responses, autophagy, and apoptosis-related pathways [108]. Therefore, AR-12 should be interpreted as a multifunctional compound with reported PDK1 activity rather than as a selective PDK1 inhibitor. In selected head-and-neck-cancer models, PDK1 suppression and kinase-inactive PDK1 experiments provided evidence supporting a contribution of PDK1 signaling to AR-12-mediated effects. However, these findings are model-specific and do not establish that the antitumor activity of AR-12 across cancer types is predominantly mediated through PDK1.

AR-12/OSU-03012 has undergone a first-in-human Phase I study in patients with advanced or recurrent solid tumors or lymphoma [109]. This study established human exposure and dose-related information but did not demonstrate PDK1-specific target engagement or clinical efficacy. Therefore, AR-12 exposure in humans should not be considered evidence of PDK1-specific clinical validation

5.2.1.2. PIF-pocket ligands and allosteric modulators. The PIF pocket is a regulatory docking site within the kinase domain of PDK1. It binds to phosphorylated hydrophobic motifs found in certain AGC-family substrates, such as S6K, SGK, and RSK, and helps to dock and organize substrates for catalysis. Importantly, the PIF pocket itself is not a phosphorylation site. The compounds directed by the PIF pocket are mechanistically diverse and can either stabilize catalytically favorable PDK1 conformations or disrupt the binding of PIF-pocket-dependent substrates. They should not therefore be considered as a single class of agonists or inhibitors [110].

PS48 is a well-characterized PIF-pocket-binding allosteric activator of PDK1. Biochemical and structural studies have revealed that PS48 binds to the PIF/hydrophobic-motif pocket and stabilizes a catalytically favorable conformation of PDK1. Therefore, PS48 is not a PDK1 inhibitor, but an allosteric agonist. Its effects on downstream substrates depend on the experimental system and should not be taken as proof of therapeutic efficacy of PIF-pocket inhibition. PS210 is also an allosteric activator of PDK1 in biochemical assays. The binding of PS210 to the PIF pocket induces a conformation of the kinase that is catalytically favorable. But at the same time, binding to the PIF pocket can also block the binding of substrates that require direct interaction with this regulatory site. Therefore, allosteric activation of PDK1 catalytic competence and inhibition of PIF-pocket-dependent substrate docking need not be mutually exclusive effects. PS423, the cell-permeable di-ester prodrug of PS210, cannot be considered just a PDK1 agonist. For cellular studies, PS423 has been reported to be a substrate-selective inhibitor after intracellular conversion to PS210. In the reported models, treatment decreased phosphorylation and activation of the PIF-pocket-dependent substrate S6K, but had relatively little effect on PKB/AKT activation, as shown in Table S4. The results show that the functional consequences of PIF pocket occupancy can vary with the substrate and experimental context. Biochemically, PS210 is an allosteric activator of the PIF pocket, while PS423 is a cell-permeable prodrug that yields substrate-selective inhibition of PIF-pocket-dependent signaling in cells.

PIFtide and related PIF-motif-mimetic ligands can also regulate PDK1-PIF-pocket-dependent interactions with substrates. The effects are sequence-dependent, ligand-dependent, cell-permeable, substrate-dependent, and context-dependent. These agents should thus be classified based on their known biochemical or cellular activity, and not all should be considered as agonists or inhibitors. In general, inhibitory or substrate-docking-disrupting PIF-pocket ligands can affect the phosphorylation of specific PIF-pocket-dependent substrates without competing for ATP binding. They are ligand- and substrate-specific and cannot be said to be universally sparing AKT or to completely inhibit

non-AKT AGC-kinase signaling. Structural or biochemical binding studies, PIF-pocket mutagenesis, and substrate-specific functional experiments are the most convincing evidence for mechanistic attribution [33]. PS48 and PS210 are direct L1 mechanistic evidence as PIF-pocket-binding allosteric activators. Mechanistic evidence for PS423 comes from its relationship with PS210, and cellular evidence of substrate-selective inhibition, especially of S6K. The results, however, do not provide general proof of the efficacy of PIF-pocket modulation or its clinical usefulness. There is no strong genetic rescue, antitumor, or clinical evidence for these compounds in L3, L4, or L5.

5.2.1.3. PH-domain-directed compounds. The compounds that target the phosphoinositide-dependent membrane recruitment are not designed to compete at the catalytic ATP-binding site. These compounds may affect the colocalization of PDK1 with membrane-associated substrates like AKT by disrupting the localization of PH domain-containing proteins. PHT-427 has been reported to bind to the PH domains of both PDK1 and AKT and to decrease their membrane localization. It does not directly bind to the ATP-binding site of PDK1 and should not be called a selective catalytic PDK1 inhibitor. Reduced AKT-associated signaling, proliferation, and survival have been reported in cellular studies, and xenograft activity has been reported. Its dual activity on the PH domains of PDK1 and AKT, however, makes it difficult to attribute it to PDK1 [15]. There is limited genetic rescue and selective comparator evidence, and the available *in vivo* data do not demonstrate that the antitumor effects are specifically PDK1-mediated. PHT-427 should thus be considered a PH-domain-directed pathway modulator of PDK1-AKT membrane recruitment [111]. PHT-427 has mechanistic but non-selective L1 evidence, indirect L2 cellular evidence, and indirect L4 animal evidence. There is no validation for PDK1-specific L3 and L5.

5.2.2. Barriers to clinical development

There is no clinical validation of a selective PDK1 inhibitor in oncology. The published literature, however, does not provide a single reason for the limited clinical progression of these compounds. Biochemical selectivity, cellular target engagement, pharmacokinetic exposure, metabolic stability, tissue distribution, tolerability, tumor-context dependency, and lack of validated patient-selection biomarkers are potential challenges. The relative importance of these factors varies from compound to compound and, in many instances, is not fully reported [87]. The ATP-competitive PDK1 inhibitors generally face challenges in medicinal chemistry due to the conservation of the ATP-binding site across many protein kinases. However, compounds should not be considered to have poor permeability, high lipophilicity, metabolic instability, or inadequate oral bioavailability without experimental data from the compound. Likewise, lack of clinical development should not be taken as a sign of failure due to pharmacokinetic or safety issues [104]. Compounds in the SNS-229 and SNS-510 series exhibited favorable oral bioavailability, robust modulation of pathways, and antitumor activity in preclinical hematologic xenograft models [112]. Based on the available information, SNS-510 progressed through IND-enabling pre-clinical development but has not been reported to enter clinical evaluation, and no primary source clearly documents the specific reason for this. Potential contributing factors, such as pharmacokinetic exposure, tolerability, or internal development prioritization, remain unconfirmed rather than established, and the absence of clinical advancement should not be interpreted as evidence of target invalidity.

5.2.2.1. Metabolic stability and clearance. The level and duration of target engagement needed for a particular therapeutic approach is dependent on adequate pharmacokinetic exposure. Relevant parameters include absorption, plasma exposure, free-drug concentration, metabolic stability, clearance, tissue distribution, and tumor penetration. Exposure time may vary depending on whether a compound is a reversible catalytic inhibitor or a compound that causes long-lasting

disruption of substrate docking or protein degradation. There are still a few reported PDK1 modulators for which compound-specific pharmacokinetic data are limited. Hence, the term rapid metabolism, short half-life, or inadequate tumor exposure should be used only if directly demonstrated for the specific compound. If these data are not available, these factors should be reported as unconfirmed developmental issues, not as proven reasons for poor efficacy or clinical non-progression [11].

5.2.2.2. On-target toxicity risks. One of the challenges in developing PDK1 inhibitors is the kinase's crucial role in normal physiology. The complete deletion of PDK1 is embryonically lethal in mice, underscoring the importance of PDK1 in development and cellular homeostasis. Its removal in certain tissues is still detrimental. The tissue-specific knockout models further emphasize the importance of PDK1 in metabolic regulation and organ function and highlight the potential risk of systemic inhibition. Systemic inhibition of PDK1 could result in greater damage to gastrointestinal (GI), hepatic, and renal tissues because PDK1 is also involved in glucose metabolism and insulin signaling, as well as protein synthesis [113].

5.2.2.3. Lack of functional selectivity. Selectivity should be considered in a few ways. Biochemical selectivity refers to the activity against PDK1 compared to other kinases, while functional selectivity refers to the degree to which a compound inhibits different PDK1 substrates, cellular compartments, and signaling outputs. Off-target kinase activity may occur with ATP-site conservation, especially for broad-spectrum multi-kinase drugs like BX-795. In contrast, GSK2334470 is relatively selective in biochemical profiling, but its cellular effects remain exposure-, substrate-priming-, and tumor context-dependent [113]. In principle, a catalytic PDK1 inhibitor can decrease activation-loop phosphorylation of AKT, SGK, S6K, and RSK. The differences in downstream effects should not be attributed to an intrinsic inability of ATP-competitive inhibitors to act on non-AKT substrates. Rather, partial or incomplete suppression of signaling in cells may be due to intracellular concentration, substrate localization, priming phosphorylation, pathway feedback, and parallel signaling [114]. Compounds such as PF-5177624, PF-5168899, SNS-229 and SNS-510 remain preclinical. There is no published evidence confirming that their exclusion from clinical trials was due to selectivity, pharmacokinetic exposure, tolerability, development strategy, or other factors. These should be presented as potential reasons for discontinuing, not as confirmed reasons. Current barriers are deduced primarily from L1 biochemical evidence, L2 cellular evidence, and limited L4 animal evidence. There is insufficient PDK1-specific L5 clinical evidence to define human pharmacokinetic requirements, dose-limiting toxicities, therapeutic windows, or validated mechanisms of development failure.

5.2.2.4. Biomarker and tumor-context limitations. Another challenge for clinical evaluation is the lack of prospectively validated biomarkers. Several candidate markers have been suggested, including PDPK1 copy number, total PDK1 expression, phospho-PDK1 Ser241, PTEN status, and PIK3CA alterations and downstream phosphoproteins, but none has been validated for selecting patients for selective PDK1-directed therapy. Phospho-Ser241 is relatively stable and should not be used as a sole dynamic measure of pathway inhibition. Future studies should include composite genomic and pharmacodynamic measurements and establish tumor contexts in which PDK1 dependency is demonstrated genetically and pharmacologically.

5.2.3. Emerging strategies

Emerging approaches to modulating PDK1 include PIF-pocket-directed ligands and peptides, dual-site inhibitors, and targeted protein degradation strategies. These approaches are intended to investigate substrate docking, conformational regulation, or removal of the PDK1 protein rather than relying solely on competition at the ATP-

binding site. However, improved selectivity, pharmacokinetic properties, safety, and therapeutic efficacy have not yet been established for these strategies. The available evidence remains predominantly biochemical and cellular, with limited *in vivo* validation and no PDK1-specific clinical evidence [69].

5.2.3.1. PIF-motif-mimetic and PIF-pocket-directed peptides. PIF-motif-mimetic peptides and conformationally stabilized peptide designs have been explored as means of interacting with the PIF pocket and modifying the interaction between PDK1 and specific AGC-kinase substrates. These molecules act at a substrate-docking interface rather than at the ATP-binding site and could be used to investigate substrate-specific regulation [115]. These peptides have effects that are sequence-, affinity-, cell-uptake-, and proteolytic-stability-dependent, and also depend on the specific substrate being studied, as shown in Table S4. It is not known whether they inhibit all PIF-pocket-dependent substrates, the non-catalytic functions of PDK1, or are more selective than optimized small molecules. Therefore, claims of scaffolding function inhibition should be avoided unless supported by direct protein-interaction experiments [116]. Available evidence is mainly biochemical and cellular. Additional studies are needed to determine cell permeability, intracellular target engagement, pharmacokinetics, tumor delivery, toxicity, and *in vivo* efficacy. PIF-pocket-directed peptides offer primarily biochemical (L1) and some cellular (L2) evidence. There is no strong PDK1-specific genetic rescue, *in vivo*, or clinical evidence [10].

5.2.3.2. Dual-site inhibitors. SNS-229 and SNS-510 have been reported as dual-site PDK1 inhibitors that stabilize an inactive conformation of the kinase and disrupt the PIF-pocket interaction for substrate docking. The term "PI3K-independent substrate recruitment" should be avoided, as substrate priming, abundance, localization, and signaling inputs like mTORC1, mTORC2, and ERK remain important for the regulation of PIF-pocket-dependent substrates [114]. These compounds are proposed to affect catalytic activity and substrate docking based on biochemical, structural, and cellular studies as shown in Table S4. Biochemical and selectivity data are, however, still scarce in the public domain, and direct genetic-rescue experiments with inhibitor-resistant PDK1 have not been reported. Thus, it is unclear whether their antiproliferative activity is solely due to PDK1. SNS-510 was found to be antiproliferative in selected hematological cancer cell lines and was reported to be approximately 10–30-fold more potent than GSK2334470, particularly in 72-h viability or pathway assays. This comparison is assay-specific and does not imply general superiority over ATP-competitive inhibitors. Oral SNS-510 treatment resulted in significant tumor-growth inhibition and partial regression at higher doses in an MV4–11 acute myeloid leukemia (AML) xenograft model. The *in vivo* evidence, however, comes mainly from a single hematological xenograft model. The results should not be extrapolated to solid tumors, immunocompetent models, or clinical efficacy until further independent validation. Evidence profile: SNS-229 and SNS-510 have L1 evidence with limited publicly available mechanistic detail and L2 cellular evidence. SNS-510 is supported by L4 evidence from the MV4–11 xenograft model, but lacks L3 rescue evidence from PDK1 and L5 clinical validation [117].

5.2.3.3. PROTAC mediated PDK1 degradation. Proteolysis-targeting chimeras and related targeted-degradation technologies are conceptually distinct from reversible catalytic inhibition. In principle, degradation of the PDPK1-encoded PDK1 could lead to decreased catalytic activity and the loss of protein surfaces involved in substrate docking or other reported interactions. But the removal of these functions should not be taken for granted without direct experimental evaluation. There is limited evidence of selective degradation of PDPK1-encoded PDK1 protein. Existing studies report short-term changes in protein abundance and downstream signaling, mostly under specific experimental conditions where degradation has been reported [61]. There is no evidence of

durable suppression of AKT Thr308, SGK-associated signaling, or other downstream nodes, and comparative evidence against ATP-competitive inhibitors is still lacking. It has also not been shown to have better antitumor activity, better tumor selectivity, or a broader therapeutic window when degraded [118]. Degradation potency and maximum degradation, proteasome and E3-ligase dependence, kinome and proteome selectivity, PDK1-specific rescue, pharmacokinetic and pharmacodynamic relationships, tumor penetration, normal-tissue effects, and comparison with catalytic inhibitors should be rigorously evaluated. Targeted degradation of PDK1-encoded PDK1 should be considered an early preclinical or investigational approach at this time. There is no evidence available to confirm long-term suppression of the pathway, widespread *in vivo* efficacy, or L5 clinical validation [62].

5.3. Polypharmacology versus selective targeting in PDK1 inhibition

Modulation of PDK1-associated signaling by pharmacological agents requires a balance between target selectivity and pathway activity. Highly selective compounds can aid mechanistic interpretation and may minimize some off-target effects, but their cellular efficacy is dependent on tumor dependency, intracellular exposure, substrate regulation, and pathway feedback [119]. On the other hand, multikinase compounds and natural products can target multiple signaling pathways, which makes it difficult to assign their biological effects specifically to PDK1. However, broader pathway modulation is not necessarily a therapeutic benefit as it can also lead to off-target effects, toxicity, and pharmacological uncertainty. Likewise, the inhibition of PDK1 should not be assumed to induce compensatory resistance in all tumor settings. Combination strategies with PDK1 and parallel signaling nodes are therefore testable preclinical strategies that need to be assessed on a case-by-case basis for efficacy, selectivity, and tolerability [120].

5.4. Natural products targeting the PDK1 signaling axis

The PI3K-AKT-mTOR and related signaling pathways have been reported to be altered by natural products and natural product-derived compounds in cancer models. But a decrease in AKT Thr308 phosphorylation or a change in downstream mTOR/S6K signaling does not necessarily indicate direct PDK1 inhibition [121]. These changes can be due to effects on receptor tyrosine kinases, PI3K, PTEN, AMPK, AKT, mTOR, oxidative stress pathways, and other molecular targets [172,173]. In this review, compounds are classified based on the evidence of direct PDK1 engagement. Direct PDK1 inhibition requires purified-enzyme activity, validated biophysical binding, structural evidence, or another orthogonal target-engagement method. The compounds mentioned below are thus categorized as: (i) natural-product derived kinase inhibitors with direct, but potentially non-selective, PDK1 evidence; (ii) compounds with partial or unresolved PDK1 evidence; and (iii) indirect or pleiotropic PI3K-AKT pathway modulators [122].

5.4.1. Natural-product-derived compounds with direct or partial PDK1 evidence

To date, only a small number of compounds of natural or natural origin (derived from nature) have been found to directly inhibit PDK1's (biochemical/structural) activity [123].

5.4.1.1. UCN-01 (7-hydroxystaurosporine). UCN-01 is a staurosporine derivative that has direct structural and biochemical evidence of binding to the PDK1 kinase domain. Direct PDK1 inhibition is supported by crystallographic studies and purified-enzyme assays, with a reported biochemical IC_{50} in the 33 nM range. These results are Level 1 evidence of PDK1 engagement [121].

In several cancer models, reduced AKT Thr308 phosphorylation, altered downstream AGC-kinase signaling, cell-cycle arrest, and

apoptosis have been reported in cellular studies. However, UCN-01 also inhibits CHK1, PKC isoforms, CDKs, and other kinases. Thus, its cellular and antitumor effects cannot be solely attributed to PDK1 without target-specific rescue or inhibitor-resistant PDK1 experiments [124]. UCN-01 has shown antitumor activity in some xenograft models and has been tested in Phase I and Phase II clinical trials (Table S5), usually in combination with other drugs. These human studies provide clinical exposure to UCN-01, but are not clinical validation of PDK1. L1 confirmed, but non-selective; L2 supported, but pharmacologically confounded; L3 incomplete due to lack of PDK1-specific rescue; L4 indirect; human exposure available, but no PDK1-specific L5 validation [125].

5.4.1.2. Neoalbaconol. Neoalbaconol is a fungal natural product isolated from *Albatrellus confluens*. Reduced AKT Thr308 phosphorylation, changes in PI3K-AKT-HK2-associated signaling, energy depletion, and induction of apoptosis or necroptosis have been reported in selected nasopharyngeal and other cancer-cell models [126]. The available study also proposed interaction with PDK1 by computational docking. Docking is a structural hypothesis and does not prove direct binding or enzymatic inhibition. If a purified PDK1 kinase assay was performed, the assay format, quantitative potency, controls, and selectivity should be explicitly reported. Neoalbaconol should not be stated to be a confirmed direct PDK1 inhibitor without quantitative biochemical confirmation [127]. The effects of AKT overexpression on selected metabolic and cell-death effects were reported to be reduced, suggesting that the effects were not direct on PDK1, but rather on the broader AKT-associated pathway. Genetic rescue of PDK1, reconstitution of inhibitor-resistant PDK1, and kinome-wide selectivity profiling have not been demonstrated. Xenograft studies provide evidence of antitumor activity for L4, but the PDK1-specific contribution to the *in vivo* response remains unresolved. There is no evidence for L3 PDK1 and indirect evidence for L4 PDK1, and no clinical evidence for L5 PDK1 [3].

5.4.2. Indirect PI3K/AKT pathway modulators

A number of natural compounds, such as quercetin, curcumin, resveratrol, berberine, luteolin, genistein, apigenin, baicalein, and emodin, have been reported to inhibit PI3K-AKT-mTOR-associated signaling in cancer models. But decreased AKT Thr308 phosphorylation or downstream mTOR/S6K activity is not sufficient to confirm direct PDK1 inhibition, as these compounds also target receptor tyrosine kinases, PI3K, PTEN, AMPK, NF- κ B, MAPK, and other signaling pathways. These compounds are not considered direct PDK1 inhibitors but rather indirect or pleiotropic pathway modulators, in the absence of validated PDK1 binding, purified enzyme inhibition, or orthogonal intracellular target engagement. Mechanisms, pharmacological limitations, and evidence profiles are detailed for each compound in Fig. 6 and Table S6 [128].

5.4.3. Combination strategies targeting PDK1 signaling

Combination approaches involving PDK1-associated signaling have been investigated with mTOR, AKT, PI3K, proteasome, cytotoxic, and radiation-based interventions. These combinations differ substantially in pharmacological selectivity and evidence strength. Some are supported by cellular and animal studies, whereas others are based mainly on pathway rationale or limited cell-based observations [129]. In several cases, the specific contribution of PDK1 cannot be separated from the activity of the partner treatment or from off-target effects of the proposed PDK1 modulator. The combinations described below should therefore be regarded as preclinical strategies rather than clinically validated therapeutic approaches. Detailed evidence levels and study limitations are summarized in Supplementary Table S4 [130].

5.4.3.1. GSK2334470 combined with PP242. GSK2334470 is an ATP-competitive PDK1 inhibitor, whereas PP242 is an ATP-competitive

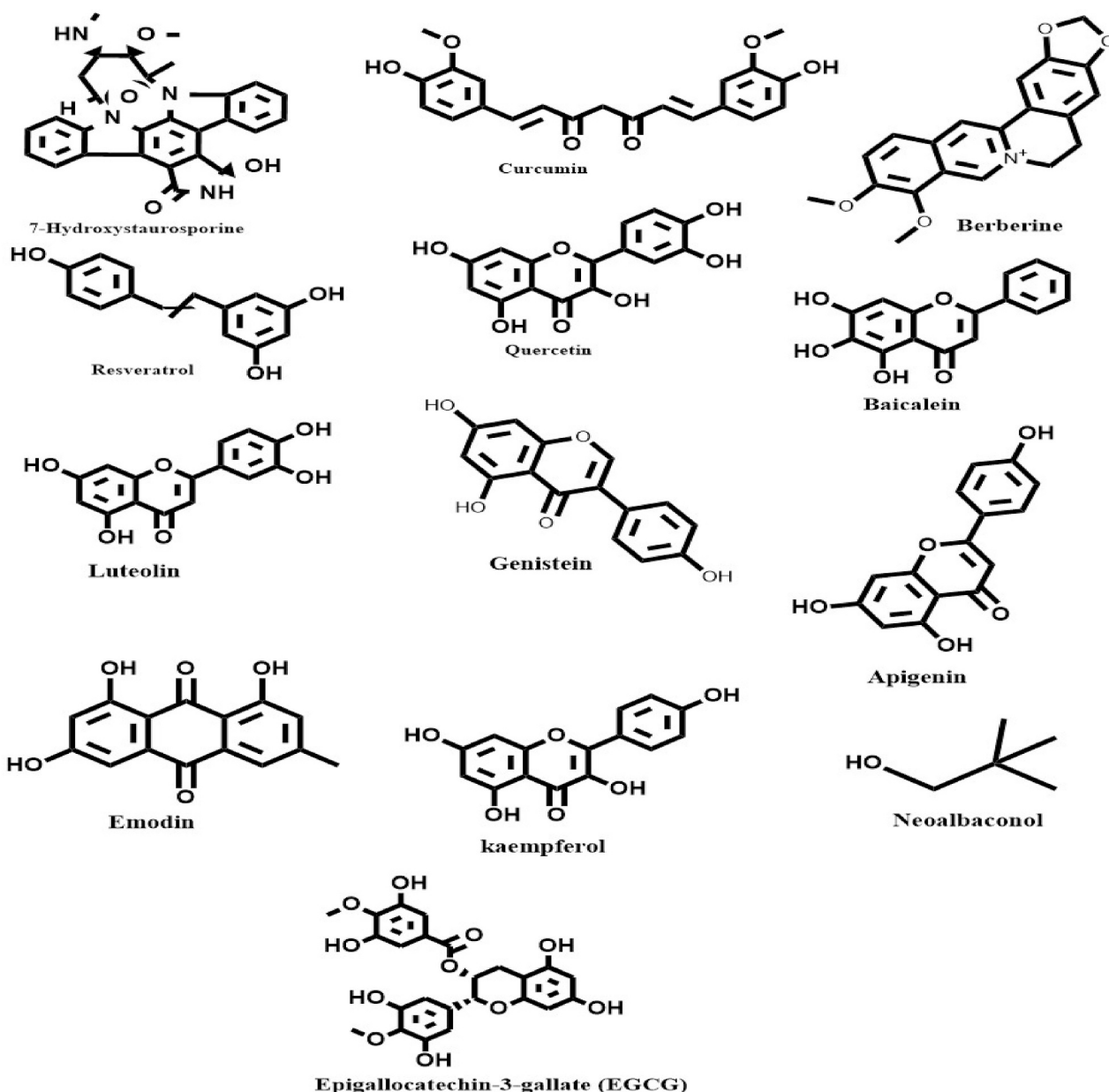


Fig. 6. Representative natural products that modulate PDK1-associated PI3K/AKT signaling. The compounds shown are predominantly indirect or pleiotropic pathway modulators; validated direct inhibition of PDK1 has not been established for most of these agents.

mTOR kinase inhibitor that inhibits both mTORC1 and mTORC2. The combination of these compounds has been explored to inhibit PDK1-dependent AKT Thr308 phosphorylation, as well as mTORC1-associated signaling and mTORC2-mediated AKT Ser473 phosphorylation. In multiple-myeloma cell lines RPMI-8226 and ARP-1, the combination inhibited the phosphorylation of AKT Thr308 (a direct target of PDK1) and Ser473 (mTORC2-dependent) more effectively than either compound alone, and also reduced the phosphorylation of p70S6K at Thr389 (the hydrophobic-motif site; not the PDK1-direct activation-loop site Thr229) and 4E-BP1 at Thr37/46 (mTORC1-dependent), while increasing cell death compared to either compound alone [118]. The combination was also found to be more effective at inhibiting tumor growth in an RPMI-8226 xenograft model, which was chosen because this line is relatively resistant to both GSK2334470 and PP242 alone.

In this study, PTEN status was correlated with sensitivity to GSK2334470 monotherapy. The ARP-1 and MM.1R cells with higher PTEN levels were more sensitive to single-agent GSK2334470, with IC50

values of 3.98 and 4.89 μ M, respectively, whereas the PTEN-deficient RPMI-8226 and OPM-2 cells were relatively resistant, with IC50 values of 8.4 and 10.56 μ M, respectively [118]. The growth-inhibitory effect of GSK2334470 was partially attenuated by PTEN knockdown, whereas PTEN overexpression increased cell sensitivity to GSK2334470. These results do not imply that PTEN is a clinically validated predictive biomarker or that PTEN-dependent modulation of the response to GSK2334470 monotherapy is a genetic rescue in the experimental models used. In contrast, the combination of GSK2334470 + PP242 was tested in the relatively GSK2334470-resistant RPMI-8226 cells and the more sensitive ARP-1 cells, and resulted in higher cytotoxicity than either agent alone [118]. Importantly, the original study included a formal drug-interaction analysis using the Chou–Talalay method and the software CalcuSyn. Isobologram analysis showed good synergism, with reported combination index (CI) values <0.4 . For instance, in RPMI-8226 cells, 2 μ M GSK2334470 and 2 μ M PP242 alone reduced viability to 82.11% and 65.08%, respectively, whereas the combination

reduced viability to 45.5% [118]. These results suggest synergy, not just additivity, in the experiments. The authors also noted combination activity in both GSK2334470-sensitive and relatively resistant myeloma models, indicating that the combination activity is less dependent on PTEN status than is GSK2334470 monotherapy. This observation is not meant to suggest prospective validation of PTEN-independent efficacy or of PTEN as a predictive biomarker for the combination as shown in Table S7. *In vivo*, combined treatment with GSK2334470 and PP242 resulted in significantly greater inhibition of RPMI-8226 xenograft growth than either agent alone and was associated with increased apoptosis and greater suppression of mTORC1/2-associated phosphorylation readouts [118]. There is no report of formal rescue with an inhibitor-resistant PDK1 construct. GSK2334470 has not been clinically evaluated as a selective PDK1-directed anticancer agent, nor has the combination of GSK2334470 and PP242 been clinically evaluated. Thus, the combination has L2 cellular evidence, including formal CI-based synergy analysis, and selected L4 xenograft support; PDK1-specific L3 target-rescue evidence and L5 clinical validation are missing [118,131].

5.4.3.2. GSK2334470 + MG-132 combination. GSK2334470 has been evaluated with the experimental proteasome inhibitor MG-132 in multiple myeloma cell models. The combination was associated with reduced AKT-related signaling, increased nuclear PTEN accumulation, and greater antiproliferative or pro-apoptotic activity than either treatment alone [62]. The mechanism responsible for nuclear PTEN accumulation remains unresolved. The available studies did not report formal synergy analysis using a combination index, Bliss independence, or a related framework. PDK1-specific genetic phenocopy and inhibitor-resistant rescue were also not performed. No formal animal study has validated this combination using PDK1-specific pharmacodynamic endpoints. In addition, MG-132 is an experimental proteasome inhibitor with broad cellular effects; therefore, the results should not be generalized to clinically used proteasome inhibitors without direct experimental evidence. Limited L2 cellular evidence is available. Combination-specific L3, L4, and L5 evidence is absent [132].

5.4.3.3. BX-795 + cisplatin combination/radiotherapy. BX-795 is a multi-kinase inhibitor with biochemical activity against PDK1, TBK1, IKK ϵ , NIAK1, and several additional kinases. In oral squamous cell carcinoma models, BX-795 combined with cisplatin or radiotherapy was associated with reduced proliferation, altered glycolytic and CD47/AKT-associated pathway readouts, changes in epithelial-mesenchymal transition-associated markers, and enhanced cell death compared with the individual treatments. Formal combination-index or Bliss-independence analyses were not consistently reported. More importantly, the effects cannot be attributed specifically to PDK1 because the studies did not include a selective PDK1 comparator, a PDK1 genetic phenocopy, an inhibitor-resistant rescue, or parallel controls for TBK1 and IKK ϵ . Any animal findings should therefore be described as evidence for a BX-795-containing combination rather than as validation of a PDK1-directed combination strategy. Indirect L2 cellular and limited L4 preclinical evidence is available. PDK1-specific L3 and L5 evidence are absent [133].

5.4.3.4. PDK1 Inhibitor + PI3K γ inhibitor combination. Combined modulation of PDK1 and PI3K γ has been investigated in selected KRAS-driven cancer models to reduce parallel or compensatory signaling in the PI3K pathway. Cellular and three-dimensional organoid studies reported greater suppression of pathway activity and tumor-cell growth with combined intervention than with individual intervention [117]. Formal synergy analysis using combination index or Bliss independence methods was not reported. Genetic deletion of PDK1 in related KRAS-driven models provides separate L3 evidence that PDK1 can contribute to tumor-cell survival; however, genetic deletion does not establish the selectivity or synergy of the pharmacological combination. Evidence for

the exact drug combination in animal models remains limited and should be distinguished from broader findings obtained using genetic models or related pathway interventions. The exact PDK1 inhibitor and PI3K γ inhibitor should be named in the text and table. When the primary source does not clearly identify both compounds, the strategy should be described as combined pathway intervention rather than as a defined drug combination. L2 cellular or organoid evidence and partial L3 genetic support are available. Combination-specific L4 evidence is limited, and no L5 clinical validation exists [134].

5.4.3.5. GSK2334470 + MK-2206 combination. GSK2334470 inhibits PDK1 catalytic activity, whereas MK-2206 is an allosteric AKT inhibitor. The combination has been evaluated to suppress PDK1-mediated AKT Thr308 phosphorylation together with AKT catalytic signaling [135]. In selected NOTCH1-mutant head-and-neck-squamous-cell-carcinoma models, the combination produced a greater reduction of AKT-associated pathway readouts and greater apoptosis than either compound alone. However, formal synergy analysis was not reported, and no PDK1 knockdown, inhibitor-resistant rescue, or animal combination experiment was included. The association with NOTCH1 mutation should therefore be considered an exploratory model-specific observation rather than a validated patient-selection biomarker. Although MK-2206 has undergone clinical evaluation in other contexts, the combination has not been evaluated clinically as a PDK1-directed strategy. L2 cellular evidence is available. Combination-specific L3, L4 and L5 evidence is absent [136].

5.4.3.6. AR-12 (OSU-03012) + Cetuximab combination. AR-12/OSU-03012 is a pleiotropic investigational compound that affects PDK1-associated survival signaling together with endoplasmic reticulum stress, chaperone, autophagy, and other cellular pathways. Its combination with cetuximab has been evaluated in head-and-neck-squamous-cell-carcinoma models to determine whether broader survival-pathway modulation enhances response to EGFR inhibition [137]. Cellular and xenograft studies reported greater inhibition of proliferation or tumor growth with the combination than with either treatment alone. RNA-interference and kinase-inactive PDK1 experiments provide partial support for a PDK1-associated contribution in selected HNSCC models, but they do not establish that the combination effect is predominantly or exclusively PDK1-mediated.

AR-12 has undergone a first-in-human Phase I study, but that trial did not establish PDK1-specific target engagement or clinical efficacy. The AR-12–cetuximab combination has not been clinically validated as a PDK1-directed strategy [138]. Indirect L2 cellular, partial L3 mechanistic, and indirect L4 xenograft evidence is available. Human exposure to AR-12 does not constitute PDK1-specific L5 validation.

5.4.3.7. PI3K α inhibitor + PDK1/SGK inhibitor combination. Alpelisib, formerly designated BYL719, is a PI3K α inhibitor. In selected breast cancer models, prolonged PI3K α inhibition has been associated with the persistence or reactivation of PDK1-regulated SGK1/SGK3 and mTORC1-associated signaling. These findings have motivated the evaluation of PI3K α inhibition in combination with separate downstream interventions [9]. The interventions must be distinguished mechanistically. Catalytic PDK1 inhibition with GSK2334470, direct pharmacological SGK inhibition, genetic PDK1 depletion, and genetic depletion of individual SGK isoforms affect different molecular targets and should not be grouped as one “PDK1/SGK-axis inhibitor” class. In selected experimental models, suppression of PDK1 or SGK signaling reduced residual SGK–mTORC1-associated outputs and increased sensitivity to PI3K α inhibition [139]. Genetic depletion of SGK isoforms supports an SGK-associated resistance mechanism but does not independently validate a pharmacological PDK1 combination. Evidence remains primarily cellular and genetic, with limited combination-specific animal validation. L2 cellular and partial L3 genetic evidence support the proposed

resistance mechanism in selected breast-cancer models. Robust L4 combination evidence and PDK1-specific L5 clinical validation are absent [140].

Overall, combination studies indicate that PDK1-associated signaling can interact with mTOR, AKT, PI3K, proteasome, and treatment-response pathways in selected experimental models. However, few combinations include formal synergy analysis, PDK1-specific rescue, comprehensive pharmacodynamic assessment, or independent *in vivo* validation. None has been clinically validated as a selective PDK1-directed combination. These findings should therefore be interpreted as preclinical evidence-generating studies rather than established therapeutic strategies.

5.5. Limitations and clinical prospects of PDK1-targeted therapies

PDK1 has substantial biological and preclinical relevance in several cancer models, but selective PDK1-directed therapy has not achieved clinical validation. The available evidence consists predominantly of L1 biochemical studies, L2 cellular pharmacology, L3 genetic-dependency studies and selected L4 animal experiments. No selective PDK1 inhibitor has established PDK1-specific target engagement, patient selection, and therapeutic benefit at the L5 clinical level.

The limited clinical progression of PDK1-directed compounds cannot currently be attributed to one established cause. Potential development considerations include biochemical selectivity, intracellular and tumor exposure, metabolic stability, tissue distribution, tolerability, pharmacodynamic target engagement, pathway redundancy, and tumor-context dependency. However, the contributions of these factors vary across compounds and are reported incompletely. The absence of clinical progression should therefore not be interpreted as proof of pharmacokinetic failure, unacceptable toxicity, or lack of target validity [89]. PDK1 expression, PDK1 copy-number alteration, and phospho-PDK1 Ser241 have been associated with aggressive tumor characteristics or poorer outcomes in selected retrospective studies of breast, lung, liver, gastric, esophageal, and other malignancies. These associations may support prognostic investigation, but they do not establish predictive utility for PDK1-directed treatment. Because Ser241 is largely constitutively auto-phosphorylated and required for catalytic competence, phospho-Ser241 should not be interpreted as a dynamic indicator of acute upstream PI3K activity. Increased phospho-Ser241 staining may instead reflect increased PDK1 protein abundance or the amount of catalytically competent protein [98]. A single biomarker is therefore unlikely to be sufficient for patient selection. Future studies should prospectively assess composite profiles that may include PDK1 copy number or expression, PTEN status, PIK3CA alterations, and downstream pathway readouts such as AKT Thr308, SGK-associated phosphorylation, and mTORC1-associated markers. These proposed markers remain exploratory and require standardized assays, predefined thresholds, independent validation cohorts, and correlation with direct pharmacodynamic target engagement [141]. Early clinical evaluation would also require a compound with well-characterized selectivity and exposure, paired tumor or surrogate-tissue pharmacodynamic assessment, confirmation of PDK1 pathway modulation, and predefined tumor-context or biomarker criteria. Until such studies are performed, PDK1 should be regarded as a biologically supported but clinically unvalidated therapeutic target [142].

6. Future perspectives

The evidence reviewed here identifies several research priorities for determining whether PDK1 can progress from a biologically supported target to a clinically validated therapeutic strategy. These priorities include improved pharmacological selectivity, the development of context-specific biomarkers, the evaluation of adaptive pathway responses, and rigorous validation of emerging approaches such as allosteric modulation and targeted protein degradation. Current evidence

remains concentrated at the L1-L4 levels, and none of these approaches has achieved PDK1-specific L5 clinical validation [143]. Computational and AI-assisted methods may support PDK1 research in specific and bounded roles. Network modeling of the PI3K–PDK1–AKT–SGK–mTORC1 axis may help prioritize combination hypotheses, identify feedback responses, and predict whether resistance is more likely to involve AKT reactivation, SGK-dependent bypass, or parallel MAPK-associated signaling. Machine-learning approaches may also assist in the early-stage evaluation of kinase selectivity, physicochemical properties, and potential off-target activity during inhibitor design. These methods should be regarded as hypothesis-generating tools rather than substitutes for biochemical and experimental validation. Predictions should be tested using defined genetic and disease contexts, including selected breast-cancer, melanoma, and osimertinib-resistant non-small-cell-lung-cancer models. Validation should include direct target-engagement measurements, pathway-specific pharmacodynamic endpoints, genetic perturbation, and appropriate *in vivo* models [9,144].

ATP-competitive inhibitors directly suppress PDK1 catalytic activity but are not designed to disrupt protein–protein interfaces or substrate-docking interactions. Whether reported non-catalytic PDK1 functions remain active during catalytic inhibition, and whether this contributes to limited efficacy in particular tumor models, requires direct experimental investigation. PIF-pocket-directed ligands, substrate-docking modulators, and dual-site inhibitors therefore represent early strategies for investigating functional selectivity beyond the ATP-binding site.

SNS-510 provides cellular evidence and selected L4 *in vivo* support for a dual-site approach. It was reported to produce greater than 95% tumor growth inhibition in an MV4–11 acute myeloid leukemia xenograft model and to be 10–30-fold more potent than GSK2334470 in selected 72-hour cellular assays. These comparisons are assay- and model-specific. Because the *in vivo* evidence derives primarily from one hematological xenograft model, it should not be generalized to solid tumors, immunocompetent systems, or clinical efficacy.

Future studies should define direct biochemical potency, binding mode, kinase selectivity, intracellular target engagement, pharmacokinetic–pharmacodynamic relationships, and activity in independently validated models. Allosteric or dual-site approaches should therefore be described as promising medicinal-chemistry directions rather than as the best or clinically preferred strategy [112,145].

Future biomarker studies should evaluate composite profiles rather than relying on a single marker. Candidate components may include PDK1 copy number or expression, PIK3CA alterations, PTEN status, ERBB2 amplification, and downstream pharmacodynamic readouts such as AKT Thr308 phosphorylation, SGK-associated signaling, and mTORC1-associated markers.

Phospho-PDK1 Ser241 should not be described as a real-time indicator of upstream pathway activation. Ser241 is largely constitutively auto-phosphorylated and is required for catalytic competence; therefore, increased tumor phospho-Ser241 staining may reflect increased abundance of catalytically competent PDK1 rather than acute PI3K-pathway activation. Its value as part of a composite biomarker panel remains exploratory [15]. Candidate biomarker panels require standardized assays, predefined thresholds, independent validation cohorts, and prospective correlation with pharmacodynamic target engagement and treatment response. Retrospective association with prognosis does not establish predictive utility of PDK1-directed therapy [9].

SGK-dependent, mTORC1-associated, and MAPK-associated adaptive signaling has been reported following pathway intervention in selected experimental models. These findings suggest that single-agent PDK1 inhibition may be insufficient in some tumor contexts, but they do not establish that resistance will occur universally. Future combination studies should be based on a defined tumor genotype, demonstrated pathway dependency, and a clearly specified pharmacological intervention. Catalytic PDK1 inhibition, direct SGK inhibition, PI3K α inhibition, mTOR inhibition, and genetic PDK1 depletion should not be

treated as interchangeable approaches. Combination experiments should include formal synergy analysis, target-specific genetic phenocopy or rescue, pharmacodynamic confirmation in tumor tissue, and comparison with appropriate single-agent controls [146]. Potential combinations with PI3K α -, mTOR-, AKT- or MAPK-directed interventions should remain preclinical hypotheses until independently validated. Immune-checkpoint combinations should likewise be presented as exploratory unless supported by direct evidence involving the PDPK1-encoded PDK1 protein in immunocompetent cancer models [119].

Targeted protein degradation represents a conceptually distinct but early strategy for investigating PDK1 biology. In principle, removal of the PDPK1-encoded PDK1 protein could suppress catalytic activity and eliminate protein surfaces involved in substrate docking or other interactions. However, loss of these functions should not be assumed solely from reduced protein abundance. Evidence for selective degradation of PDPK1-encoded PDK1 remains limited [147]. Validation of a genuine PDK1-directed degrader should include concentration- and time-dependent degradation; determination of degradation potency and maximum degradation; proteasome and E3-ligase dependence; proteome-wide selectivity; PDK1-specific rescue; comparison with catalytic inhibitors; pharmacokinetic and pharmacodynamic studies; tumor penetration; and evaluation of normal-tissue effects. Until these requirements are met, targeted PDK1 degradation should be regarded as an early medicinal-chemistry objective rather than a clinically ready strategy.

Overall, future PDK1 research should prioritize experiments that directly connect compound exposure, target engagement, downstream pathway modulation, and antitumor response in molecularly defined models. Progression toward clinical evaluation will require a selective compound, a prospectively defined patient-selection strategy, and direct demonstration that therapeutic effects arise from modulation of the PDPK1-encoded PDK1 protein [148].

7. Conclusion

PDK1 is a central regulator of AGC-family kinase signaling. In the canonical PI3K pathway, PIP3-dependent membrane recruitment facilitates PDK1-mediated phosphorylation of AKT at Thr308. PDK1 also phosphorylates the activation loops of SGK, S6K, RSK, and PKC-family kinases, although regulation of these substrates generally depends on substrate priming, localization, and PIF-pocket interactions rather than a uniform PI3K-dependent membrane-recruitment mechanism. Genetic and pharmacological studies support context-dependent contributions of PDK1 to tumor-cell survival, proliferation, metabolic adaptation, invasion, and treatment response in selected experimental models. Nevertheless, the therapeutic evidence remains concentrated at the L1 biochemical, L2 cellular, and L3 genetic levels, with selected L4 animal studies. No selective PDK1-directed intervention has established PDK1-specific target engagement, biomarker-defined patient selection, and clinical benefit at the L5 level.

The available pharmacological approaches should not be regarded as a single evidence class. GSK2334470 provides comparatively selective evidence of catalytic inhibition, whereas BX-795, AR-12/OSU-03012, and PHT-427 have multi-kinase, pleiotropic, or non-catalytic mechanisms that complicate PDK1-specific attribution. PIF-pocket ligands are also mechanistically heterogeneous and include allosteric agonists as well as substrate-docking modulators. Dual-site compounds such as SNS-510 have selected cellular and xenograft support, but their reported potency and efficacy remain assay- and model-specific. Emerging approaches involving substrate-docking modulation and targeted protein degradation require further biochemical, genetic, and *in vivo* validation. Evidence for a selective degrader of the PDPK1-encoded PDK1 protein remains limited, and the A04 degrader cannot be included because it targets the unrelated mitochondrial enzyme pyruvate dehydrogenase kinase 1. It has not been demonstrated that PDK1 degradation produces

lasting pathway suppression, broader antitumor activity, or a superior therapeutic window compared with catalytic inhibition. Biomarker development is similarly preliminary. PDK1 copy number or expression, PTEN status, PIK3CA alterations, and downstream phosphoproteins may help define tumor contexts for future investigation, but none has been prospectively validated for selecting patients for PDK1-directed treatment. Because Ser241 is largely constitutively auto-phosphorylated, phospho-PDK1 Ser241 should not be interpreted as a dynamic indicator of acute PI3K-pathway activation. Composite biomarker approaches therefore remain exploratory.

Overall, PDK1 is a biologically supported but clinically unvalidated anticancer target. Progress toward clinical evaluation will require selective, well-characterized compounds; direct pharmacodynamic evidence of PDK1 engagement; molecularly defined tumor contexts; prospectively validated biomarkers; and demonstration of an acceptable therapeutic window. Until these requirements are met, claims concerning clinical utility, precision-oncology application, or resistance reversal should remain confined to the level supported by the available preclinical evidence.

List of abbreviations

AGC	Protein kinase A, G, and C family
AKT (PKB)	Protein kinase B
AML	Acute myeloid leukemia
APC	Adenomatous polyposis coli
BAD	Bcl-2-associated agonist of cell death
BAX	Bcl-2-associated X protein
CDK	Cyclin-dependent kinase
CRISPR	Clustered regularly interspaced short palindromic repeats
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
FOXO	Forkhead box O transcription factor
HIF-1 α	Hypoxia-inducible factor-1 alpha
mTOR	Mammalian target of rapamycin
PDK1	3-Phosphoinositide-dependent protein kinase-1
PH domain	Pleckstrin homology domain
PI3K	Phosphoinositide 3-kinase
PIF pocket	PDK1-interacting fragment pocket
PKC	Protein kinase C
PROTAC	Proteolysis-targeting chimera
PTEN	Phosphatase and tensin homolog
RTK	Receptor tyrosine kinase
SGK	Serum- and glucocorticoid-regulated kinase
S6K	Ribosomal protein S6 kinase
VEGF	Vascular endothelial growth factor

CRediT authorship contribution statement

Darshana Ballagere Honnasiddappa: Writing – original draft, Investigation, Conceptualization. **Mohankumar Ramar:** Writing – original draft, Formal analysis. **Tincy Biju:** Writing – original draft, Investigation. **Mallikarjun Sajjan:** Writing – original draft, Formal analysis. **Chidananda Venkatesh:** Writing – original draft, Formal analysis. **Nayan Kumar Mahadeva:** Writing – original draft, Formal analysis. **Selvaraj Kunjiappan:** Writing – original draft, Supervision. **Damodar Nayak Ammunje:** Writing – review & editing, Supervision, Conceptualization. **Parasuraman Pavadai:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

The authors thank M.S. Ramaiah University of Applied Sciences, Bengaluru, India, for supporting this project by providing the necessary facilities.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not Applicable.

Funding

This research received no external funding.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2026.154405>.

Data availability

The manuscript files include all the data generated during the study.

References

- [1] J.R. Medina, Selective 3-phosphoinositide-dependent kinase 1 (PDK1) inhibitors: dissecting the function and pharmacology of PDK1: miniperspective, *J. Med. Chem.* 56 (7) (2013) 2726–2737.
- [2] Q. Jiang, X. Zhang, X. Dai, S. Han, X. Wu, L. Wang, W. Wei, N. Zhang, W. Xie, J. Guo, S6K1-mediated phosphorylation of PDK1 impairs AKT kinase activity and oncogenic functions, *Nat. Commun.* 13 (1) (2022) 1548.
- [3] C. Raimondi, M. Falasca, Targeting PDK1 in cancer, *Curr. Med. Chem.* 18 (18) (2011) 2763–2769.
- [4] O.A. Bamodu, H.-L. Chang, J.-R. Ong, W.-H. Lee, C.-T. Yeh, J.-T. Tsai, Elevated PDK1 expression drives PI3K/AKT/mTOR signaling promotes radiation-resistant and dedifferentiated phenotype of hepatocellular carcinoma, *Cells* 9 (3) (2020) 746.
- [5] N. Wang, J. Fu, Z. Li, N. Jiang, Y. Chen, J. Peng, The landscape of PDK1 in breast cancer, *Cancers* 14 (3) (2022) 811.
- [6] L. Di Blasio, P.A. Gagliardi, A. Puliafito, L. Primo, Serine/threonine kinase 3-phosphoinositide-dependent protein kinase-1 (PDK1) as a key regulator of cell migration and cancer dissemination, *Cancers* 9 (3) (2017) 25.
- [7] S. Algheribe, A. Zagzoog, M. Boudjelal, I. Islam, Phosphoinositide-dependent kinase 1 (PDK1) in cancer: molecular insights and therapeutic strategies, *Front. Chem.* 14 (2026) 1767926.
- [8] X. Xu, Y. Chen, Q. Fu, D. Ni, J. Zhang, X. Li, S. Lu, The chemical diversity and structure-based discovery of allosteric modulators for the PIF-pocket of protein kinase PDK1, *J. Enzyme Inhib. Med. Chem.* 34 (1) (2019) 361–374.
- [9] P. Castel, H. Ellis, R. Bago, E. Toska, P. Razavi, F.J. Carmona, S. Kannan, C. S. Verma, M. Dickler, S. Chandralapaty, PDK1-SGK1 signaling sustains AKT-independent mTORC1 activation and confers resistance to PI3K α inhibition, *Cancer Cell* 30 (2) (2016) 229–242.
- [10] M. Sacerdoti, L.Z. Gross, A.M. Riley, K. Zehnder, A. Ghode, S. Klinke, G.S. Anand, K. Paris, A. Winkel, A.K. Herbrand, Modulation of the substrate specificity of the kinase PDK1 by distinct conformations of the full-length protein, *Sci. Signal.* 16 (789) (2023) eadd3184.
- [11] M. Sirico, A. D'Angelo, C. Gianni, C. Casadei, F. Merloni, U. De Giorgi, Current state and future challenges for PI3K inhibitors in cancer therapy, *Cancers* 15 (3) (2023) 703.
- [12] A. Sathe, G. Chalaud, I. Oppolzer, K.Y. Wong, M. von Busch, S.C. Schmid, Z. Tong, M. Retz, J.E. Gschwend, W.A. Schulz, Parallel PI3K, AKT and mTOR inhibition is required to control feedback loops that limit tumor therapy, *PLoS One* 13 (1) (2018) e0190854.
- [13] N. Zheng, J. Wei, D. Wu, Y. Xu, J. Guo, Master kinase PDK1 in tumorigenesis, *Biochim. Biophys. Acta Rev. Cancer* 1878 (6) (2023) 188971.
- [14] M. Whitman, D.R. Kaplan, B. Schaffhausen, L. Cantley, T.M. Roberts, Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation, *Nature* 315 (6016) (1985) 239–242.
- [15] D.R. Alessi, M. Deak, A. Casamayor, F.B. Caudwell, N. Morrice, D.G. Norman, P. Gaffney, C.B. Reese, C.N. MacDougall, D. Harbison, 3-phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the *Drosophila* DSTPK61 kinase, *Curr. Biol.* 7 (10) (1997) 776–789.
- [16] D.R. Alessi, M.T. Kozlowski, Q.-P. Weng, N. Morrice, J. Avruch, 3-phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylates and activates the p70 S6 kinase in vivo and in vitro, *Curr. Biol.* 8 (2) (1998) 69–81.
- [17] N. Pullen, P.B. Dennis, M. Andjelkovic, A. Dufner, S.C. Kozma, B.A. Hemmings, G. Thomas, Phosphorylation and activation of p70s6k by PDK1, *Science* 279 (5351) (1998) 707–710.
- [18] Q.-P. Weng, M. Kozlowski, C. Belham, A. Zhang, M.J. Comb, J. Avruch, Regulation of the p70 S6 kinase by phosphorylation in vivo: analysis using site-specific anti-phosphopeptide antibodies, *J. Biol. Chem.* 273 (26) (1998) 16621–16629.
- [19] D. Alessi, Discovery of PDK1, One of the Missing Links in Insulin Signal Transduction, Portland Press Ltd., 2001.
- [20] J.A. Le Good, W.H. Ziegler, D.B. Parekh, D.R. Alessi, P. Cohen, P.J. Parker, Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1, *Science* 281 (5385) (1998) 2042–2045.
- [21] L.R. Pearce, D. Komander, D.R. Alessi, The nuts and bolts of AGC protein kinases, *Nat. Rev. Mol. Cell Biol.* 11 (1) (2010) 9–22.
- [22] D.R. Alessi, S.R. James, C.P. Downes, A.B. Holmes, P.R. Gaffney, C.B. Reese, P. Cohen, Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase α , *Curr. Biol.* 7 (4) (1997) 261–269.
- [23] J.R. Bayascas, PDK1: the major transducer of PI 3-kinase actions, in: *Phosphoinositide 3-kinase in Health and Disease: Volume 1*, 2010, pp. 9–29.
- [24] R.M. Biondi, P.C. Cheung, A. Casamayor, M. Deak, R.A. Currie, D.R. Alessi, Identification of a pocket in the PDK1 kinase domain that interacts with PIP and the C-terminal residues of PKA, *EMBO J.* 19 (5) (2000 Mar 1) 979–988, <https://doi.org/10.1093/emboj/19.5.979>.
- [25] L. Stephens, K. Anderson, D. Stokoe, H. Erdjument-Bromage, G.F. Painter, A. B. Holmes, P.R. Gaffney, C.B. Reese, F. McCormick, P. Tempst, Protein kinase B kinases that mediate phosphatidylinositol 3, 4, 5-trisphosphate-dependent activation of protein kinase B, *Science* 279 (5351) (1998) 710–714.
- [26] A. Mora, D. Komander, D.M. van Aalten, D.R. Alessi, PDK1, the master regulator of AGC kinase signal transduction, *Semin. Cell Dev. Biol.* (2004) 161–170. Elsevier.
- [27] A.E. Leroux, R.M. Biondi, The choreography of protein kinase PDK1 and its diverse substrate dance partners, *Biochem. J.* 480 (19) (2023) 1503–1532.
- [28] R.M. Biondi, D. Komander, C.C. Thomas, J.M. Lizcano, M. Deak, D.R. Alessi, D. M. van Aalten, High resolution crystal structure of the human PDK1 catalytic domain defines the regulatory phosphopeptide docking site, *EMBO J.* 21 (16) (2002 Aug 15) 4219–4228, <https://doi.org/10.1093/emboj/cdf437>.
- [29] A. Casamayor, N.A. Morrice, D.R. Alessi, Phosphorylation of Ser-241 is essential for the activity of 3-phosphoinositide-dependent protein kinase-1: identification of five sites of phosphorylation in vivo, *Biochem. J.* 342 (2) (1999) 287–292.
- [30] A. Levina, K.D. Fleming, J.E. Burke, T.A. Leonard, Activation of the essential kinase PDK1 by phosphoinositide-driven trans-autophosphorylation, *Nat. Commun.* 13 (1) (2022) 1874.
- [31] G.d.l. Heras-Martínez, V. Calleja, R. Bailly, J. Dessolin, B. Larijani, J. Requejo-Isidro, A complex interplay of anionic phospholipid binding regulates 3'-phosphoinositide-dependent-kinase-1 homodimer activation, *Sci. Rep.* 9 (1) (2019) 14527.
- [32] B.J. Collins, M. Deak, V. Murray-Tait, K.G. Storey, D.R. Alessi, In vivo role of the phosphate groove of PDK1 defined by knockin mutation, *J. Cell Sci.* 118 (21) (2005) 5023–5034.
- [33] K. Busschots, L.A. Lopez-Garcia, C. Lammi, A. Stroba, S. Zeuzem, A. Piiper, P. M. Alzari, S. Neimanis, J.M. Arencibia, M. Engel, Substrate-selective inhibition of protein kinase PDK1 by small compounds that bind to the PIF-pocket allosteric docking site, *Chem. Biol.* 19 (9) (2012) 1152–1163.
- [34] X. Gao, T.K. Harris, Role of the PH domain in regulating in vitro autophosphorylation events required for reconstitution of PDK1 catalytic activity, *Bioorg. Chem.* 34 (4) (2006) 200–223.
- [35] C. Rommel, M. Camps, H. Ji, PI3K δ and PI3K γ : partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat. Rev. Immunol.* 7 (3) (2007) 191–201.
- [36] L. Xu, H. Jang, R. Nussinov, Capturing autoinhibited PDK1 reveals the linker's regulatory role, informing innovative inhibitor design, *J. Chem. Inf. Model.* 64 (19) (2024) 7709–7724.
- [37] M. Garcia-Viloca, J.R. Bayascas, J.M. Lluch, À. González-Lafont, Molecular insights into the regulation of 3-phosphoinositide-dependent protein kinase 1: modeling the interaction between the kinase and the pleckstrin homology domains, *ACS Omega* 7 (29) (2022) 25186–25199.
- [38] A.L. Shaw, J.E. Burke, Molecular insight to the role of the phosphoinositide PIP3 in regulating the protein kinases Akt, PDK1, and BTK, *Biochem. Soc. Trans.* 53 (4) (2025 Aug 29) 737–749, <https://doi.org/10.1042/BST20253059>.
- [39] V. De Rosa, F. Iommelli, C. Terlizzi, E. Leggiero, R. Camerlingo, G.G. Altobelli, R. Fonti, L. Pastore, S. Del Vecchio, Non-canonical role of PDK1 as a negative regulator of apoptosis through macromolecular complexes assembly at the ER-mitochondria interface in oncogene-driven NSCLC, *Cancers* 13 (16) (2021) 4133.
- [40] L.C. Cantley, The phosphoinositide 3-kinase pathway, *Science* 296 (5573) (2002) 1655–1657.
- [41] B.D. Manning, L.C. Cantley, AKT/PKB signaling: navigating downstream, *Cell* 129 (7) (2007) 1261–1274.
- [42] D. Hassan, C.W. Menges, J.R. Testa, A. Bellacosa, AKT kinases as therapeutic targets, *J. Exp. Clin. Cancer Res.* 43 (1) (2024) 313.
- [43] F. Fontana, G. Giannitti, S. Marchesi, P. Limonta, The PI3K/Akt pathway and glucose metabolism: a dangerous liaison in cancer, *Int. J. Biol. Sci.* 20 (8) (2024) 3113.

- [44] M. Lodeiro, M. Theodoropoulou, M. Pardo, F.F. Casanueva, J.P. Camina, C-Src regulates Akt signaling in response to ghrelin via β -arrestin signaling-independent and dependent mechanisms, *PLoS One* 4 (3) (2009) e4686.
- [45] A. Najafov, E.M. Sommer, J.M. Axten, M.P. DeYoung, D.R. Alessi, Characterization of GSK2334470, a novel and highly specific inhibitor of PDK1, *Biochem. J.* 433 (2) (2011) 357–369.
- [46] X. Zhao, S. Lu, J. Nie, X. Hu, W. Luo, X. Wu, H. Liu, Q. Feng, Z. Chang, Y. Liu, Phosphoinositide-dependent kinase 1 and mTORC2 synergistically maintain postnatal heart growth and heart function in mice, *Mol. Cell. Biol.* 34 (11) (2014) 1966–1975.
- [47] S. Sridharan, A. Basu, Distinct roles of mTOR targets S6K1 and S6K2 in breast cancer, *Int. J. Mol. Sci.* 21 (4) (2020) 1199.
- [48] M.K. Holz, B.A. Ballif, S.P. Gygi, J. Blenis, mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events, *Cell* 123 (4) (2005) 569–580.
- [49] J.M. García-Martínez, D.R. Alessi, mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1), *Biochem. J.* 416 (3) (2008) 375–385.
- [50] Y. Romeo, X. Zhang, P.P. Roux, Regulation and function of the RSK family of protein kinases, *Biochem. J.* 441 (2) (2012) 553–569.
- [51] R. Bago, E. Sommer, P. Castel, C. Crafter, F.P. Bailey, N. Shpiro, J. Baselga, D. Cross, P.A. Eyers, D.R. Alessi, The hVps34-SGK3 pathway alleviates sustained PI3K/Akt inhibition by stimulating mTORC1 and tumour growth, *EMBO J.* 35 (17) (2016) 1902–1922.
- [52] P.A. Gagliardi, L. di Blasio, L. Primo, PDK1: a signaling hub for cell migration and tumor invasion, *Biochim. Biophys. Acta* 1856 (2) (2015) 178–188.
- [53] D.K. Morrison, The 14-3-3 proteins: integrators of diverse signaling cues that impact cell fate and cancer development, *Trends Cell Biol.* 19 (1) (2009) 16–23.
- [54] L.R. Pearce, G.R. Alton, D.T. Richter, J.C. Kath, L. Lingardo, J. Chapman, C. Hwang, D.R. Alessi, Characterization of PF-4708671, a novel and highly specific inhibitor of p70 ribosomal S6 kinase (S6K1), *Biochem. J.* 431 (2) (2010) 245–255.
- [55] N. Grammatikakis, J.-H. Lin, A. Grammatikakis, P.N. Tsichlis, B.H. Cochran, p50 cdc37 acting in concert with Hsp90 is required for Raf-1 function, *Mol. Cell. Biol.* 19 (3) (1999) 1661–1672.
- [56] S. Pinner, E. Sahai, PDK1 regulates cancer cell motility by antagonising inhibition of ROCK1 by RhoE, *Nat. Cell Biol.* 10 (2) (2008) 127–137.
- [57] E. Sahai, C.J. Marshall, Differing modes of tumour cell invasion have distinct requirements for rho/ROCK signalling and extracellular proteolysis, *Nat. Cell Biol.* 5 (8) (2003) 711–719.
- [58] D.A. Fruman, H. Chiu, B.D. Hopkins, S. Bagrodia, L.C. Cantley, R.T. Abraham, The PI3K pathway in human disease, *Cell* 170 (4) (2017) 605–635.
- [59] A. Najafov, N. Shpiro, D.R. Alessi, Akt is efficiently activated by PIF-pocket-and PtdIns (3, 4, 5) P 3-dependent mechanisms leading to resistance to PDK1 inhibitors, *Biochem. J.* 448 (2) (2012) 285–295.
- [60] T.A. Yap, M.D. Garrett, M.I. Walton, F. Raynaud, J.S. de Bono, P. Workman, Targeting the PI3K–AKT–mTOR pathway: progress, pitfalls, and promises, *Curr. Opin. Pharmacol.* 8 (4) (2008) 393–412.
- [61] W. Zhou, J. Huang, C. Huang, G. Wang, X. Tu, Disruption of Autophagic flux and treatment with the PDPK1 inhibitor GSK2334470 synergistically inhibit renal cell carcinoma pathogenesis, *J. Cancer* 15 (5) (2024) 1429.
- [62] J. Zhang, C. Yang, F. Zhou, X. Chen, PDK1 inhibitor GSK2334470 synergizes with proteasome inhibitor MG-132 in multiple myeloma cells by inhibiting full AKT activity and increasing nuclear accumulation of the PTEN protein, *Oncol. Rep.* 39 (6) (2018) 2951–2959.
- [63] N. Bholia, Investigation of the Mechanisms and Therapeutic Implications of Crosstalk between G-Protein-Coupled Receptors and the Epidermal Growth Factor Receptor in HNSCC, University of Pittsburgh, 2009.
- [64] X. Wu, W. Xie, W. Wei, J. Guo, Beyond controlling cell size: functional analyses of S6K in tumorigenesis, *Cell Death Dis.* 13 (7) (2022) 646.
- [65] C. Yang, X. Huang, H. Liu, F. Xiao, J. Wei, L. You, W. Qian, PDK1 inhibitor GSK2334470 exerts antitumor activity in multiple myeloma and forms a novel multitargeted combination with dual mTORC1/C2 inhibitor PP242, *Oncotarget* 8 (24) (2017 Jun 13) 39185–39197, <https://doi.org/10.18632/oncotarget.16642>.
- [66] A. Engelsberg, Funktionelle Charakterisierung der Serum- und Glukokortikoid-induzierbaren Proteinkinase Sgk1, 2007.
- [67] A.C. Newton, Protein kinase C: perfectly balanced, *Crit. Rev. Biochem. Mol. Biol.* 53 (2) (2018) 208–230.
- [68] R. Dettori, S. Sonzogni, L. Meyer, L.A. Lopez-Garcia, N.A. Morrice, S. Zeuzem, M. Engel, A. Piiper, S. Neimanis, M. Frödin, Regulation of the interaction between protein kinase C-related protein kinase 2 (PRK2) and its upstream kinase, 3-phosphoinositide-dependent protein kinase 1 (PDK1), *J. Biol. Chem.* 284 (44) (2009) 30318–30327.
- [69] C. Raimondi, V. Calleja, R. Ferro, A. Fantin, A.M. Riley, B.V. Potter, C.H. Brennan, T. Maffucci, B. Larjani, M. Falasca, A small molecule inhibitor of PDK1/PLC γ 1 interaction blocks breast and melanoma cancer cell invasion, *Sci. Rep.* 6 (1) (2016) 26142.
- [70] J.D. Carpten, A.L. Faber, C. Horn, G.P. Donoho, S.L. Briggs, C.M. Robbins, G. Hostetter, S. Boguslawski, T.Y. Moses, S. Savage, A transforming mutation in the pleckstrin homology domain of AKT1 in cancer, *Nature* 448 (7152) (2007) 439–444.
- [71] I.M. Meraz, M. Majidi, B. Fang, F. Meng, L. Gao, R. Shao, R. Song, F. Li, Y. Lissanu, H. Chen, 3-phosphoinositide-dependent kinase 1 drives acquired resistance to osimertinib, *Commun. Biol.* 6 (1) (2023) 509.
- [72] C. Dong, J. Wu, Y. Chen, J. Nie, C. Chen, Activation of PI3K/AKT/mTOR pathway causes drug resistance in breast cancer, *Front. Pharmacol.* 12 (2021) 628690.
- [73] F. Huang, J. Cui, J. Wan, X. Yuan, Y. Zhu, X. Wu, W. Zuo, T. Zhao, SLC12A8 mediates TKI resistance in EGFR-mutant lung cancer via PDK1/AKT axis, *J. Cancer Res. Clin. Oncol.* 149 (18) (2023) 16729–16739.
- [74] M. Davies, K. Stemke-Hale, C. Tellez, T. Calderone, W. Deng, V. Prieto, A. Lazar, J. Gershenwald, G. Mills, A novel AKT3 mutation in melanoma tumours and cell lines, *Br. J. Cancer* 99 (8) (2008) 1265–1268.
- [75] A. Bellacosa, D. De Feo, A.K. Godwin, D.W. Bell, J.Q. Cheng, D.A. Altomare, M. Wan, L. Dubeau, G. Scambia, V. Masciullo, Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas, *Int. J. Cancer* 64 (4) (1995) 280–285.
- [76] P.A. Gagliardi, A. Puliafito, L. Primo, PDK1: at the crossroad of cancer signaling pathways, *Semin. Cancer Biol.* (2018) 27–35. Elsevier.
- [77] F. Skoulidis, J.V. Heymach, Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy, *Nat. Rev. Cancer* 19 (9) (2019) 495–509.
- [78] Y. Wang, L. Fu, M. Cui, Y. Wang, Y. Xu, M. Li, J. Mi, Amino acid transporter SLC38A3 promotes metastasis of non-small cell lung cancer cells by activating PDK1, *Cancer Lett.* 393 (2017) 8–15.
- [79] A.S. Clark, K.A. West, P.M. Blumberg, P.A. Dennis, Altered protein kinase C (PKC) isoforms in non-small cell lung cancer cells: PKC δ promotes cellular survival and chemotherapeutic resistance, *Cancer Res.* 63 (4) (2003) 780–786.
- [80] J. Yu, K.-S. Chen, Y.-N. Li, J. Yang, L. Zhao, Silencing of PDK1 gene expression by RNA interference suppresses growth of esophageal cancer, *Asian Pac. J. Cancer Prev.* 13 (8) (2012) 4147–4151.
- [81] M.-Q. Chen, B.-H. Xu, Y.-Y. Zhang, Analysis of prognostic factors for esophageal squamous cell carcinoma with distant organ metastasis at initial diagnosis, *J. Chin. Med. Assoc.* 77 (11) (2014) 562–566.
- [82] S. Sheng, M. Qiao, A.B. Pardee, Metastasis and AKT activation, *J. Cell. Physiol.* 218 (3) (2009) 451–454.
- [83] V. Calleja, M. Laguerre, G. de Las Heras-Martinez, P.J. Parker, J. Requejo-Isidro, B. Larjani, Acute regulation of PDK1 by a complex interplay of molecular switches, *Biochem. Soc. Trans.* 42 (5) (2014) 1435–1440.
- [84] A. Österroos, M. Kashif, C. Haglund, K. Blom, M. Höglund, C. Andersson, M. G. Gustafsson, A. Eriksson, R. Larsson, Combination screening in vitro identifies synergistically acting KP372-1 and cytarabine against acute myeloid leukemia, *Biochem. Pharmacol.* 118 (2016) 40–49.
- [85] A.C. Newton, Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm, *Biochem. J.* 370 (2) (2003) 361–371.
- [86] M. Krayem, P. Aftimos, A. Najem, T. van den Hooven, A. van den Berg, L. Hovstad-Bijl, R. de Wijn, R. Hilhorst, R. Ruijtenbeek, M. Sabbah, Kinome profiling to predict sensitivity to MAPK inhibition in melanoma and to provide new insights into intrinsic and acquired mechanism of resistance, *Cancers* 12 (2) (2020) 512.
- [87] M. Scortegagna, C. Ruller, Y. Feng, R. Lazova, H. Kluger, J.-L. Li, S.K. De, R. Rickert, M. Pelliccia, M. Bosenberg, Genetic inactivation or pharmacological inhibition of Pdk1 delays development and inhibits metastasis of BrafV600E::Pten–/–melanoma, *Oncogene* 33 (34) (2014) 4330–4339.
- [88] K.M. Moxley, L. Wang, A.L. Welm, M. Bieniasz, Short-form Ron is a novel determinant of ovarian cancer initiation and progression, *Genes Cancer* 7 (5–6) (2016) 169.
- [89] M. Maurer, T. Su, L.H. Saal, S. Koujak, B.D. Hopkins, C.R. Barkley, J. Wu, S. Nandula, D. Dutta, Y. Xie, 3-phosphoinositide-dependent kinase 1 potentiates upstream lesions on the phosphatidylinositol 3-kinase pathway in breast carcinoma, *Cancer Res.* 69 (15) (2009) 6299–6306.
- [90] H. Lin, F. Hsieh, H. Song, J. Lin, Elevated phosphorylation and activation of PDK1/AKT pathway in human breast cancer, *Br. J. Cancer* 93 (12) (2005) 1372–1381.
- [91] B.S. Taylor, N. Schultz, H. Hieronymus, A. Gopalan, Y. Xiao, B.S. Carver, V. K. Arora, P. Kaushik, E. Cerami, B. Reva, Integrative genomic profiling of human prostate cancer, *Cancer Cell* 18 (1) (2010) 11–22.
- [92] A. El Quamari, N. Barouk, G. Martens, P. Lebrun, D. Pipeleers, E. Van Obberghen, MiR-375 targets PDK1 and regulates glucose-induced biological responses in pancreatic beta-cells, *Diabetes* 57 (2008) 2708–2717.
- [93] C. Zhao, G. Zhang, J. Liu, C. Zhang, Y. Yao, W. Liao, Exosomal cargoes in oral squamous cell carcinoma: current findings and potential functions, *PeerJ* 8 (2020) e10062, <https://doi.org/10.7717/peerj.10062>.
- [94] M. Frödin, T.L. Antal, B.A. Dümmler, C.J. Jensen, M. Deak, S. Gammeltoft, R. M. Biondi, A phosphoserine/threonine-binding pocket in AGC kinases and PDK1 mediates activation by hydrophobic motif phosphorylation, *EMBO J.* 21 (20) (2002 Oct 15) 5396–5407, <https://doi.org/10.1093/emboj/cdf551>.
- [95] A. Emmanouilidi, M. Falasca, Targeting PDK1 for chemosensitization of cancer cells, *Cancers* 9 (10) (2017) 140.
- [96] J. Du, M. Yang, S. Chen, D. Li, Z. Chang, Z. Dong, PDK1 promotes tumor growth and metastasis in a spontaneous breast cancer model, *Oncogene* 35 (25) (2016) 3314–3323.
- [97] H. Li, H. Shen, P. Xie, Z. Zhang, L. Wang, Y. Yang, Z. Yu, Z. Cheng, J. Zhou, Role of long intergenic non-protein coding RNA 00152 in pancreatic cancer glycolysis via the manipulation of the microRNA-185-5p/Krüppel-like factor 7 axis, *J. Cancer* 12 (21) (2021) 6330.
- [98] J. Wang, F. Liu, P. Ao, X. Li, H. Zheng, D. Wu, N. Zhang, J. She, J. Yuan, X. Wu, Correlation of PDK1 expression with clinicopathologic features and prognosis of hepatocellular carcinoma, *Onco. Targets Ther.* (2016) 5597–5602.
- [99] J. Zabkiewicz, L. Pearn, R.K. Hills, R.G. Morgan, A. Tonks, A.K. Burnett, R. L. Darley, The PDK1 master kinase is over-expressed in acute myeloid leukemia and promotes PKC-mediated survival of leukemic blasts, *Haematologica* 99 (5) (2014) 858.
- [100] D. Gan, S. Yue, Y. Jiang, D. Zhang, H. Shi, H. Qian, T. Zhou, W. Fang, M. Yao, G. Zuo, Nucleus-located PDK1 regulates growth, invasion and migration of breast cancer cells, *Life Sci.* 253 (2020) 117722.

- [101] Y. Wei, D. Zhang, H. Shi, H. Qian, H. Chen, Q. Zeng, F. Jin, Y. Ye, Z. Ou, M. Guo, PDK1 promotes breast cancer progression by enhancing the stability and transcriptional activity of HIF-1 α , *Genes Dis.* 11 (4) (2024) 101041.
- [102] S. Sestito, S. Rapposelli, A patent update on PDK1 inhibitors (2015-present), *Expert Opin. Ther. Pat.* 29 (4) (2019) 271–282.
- [103] Y. Fu, Y. Zhu, X. Zhou, C. Xiao, P. Zheng, Y. Sun, S. Xu, Rational design of next-generation PDK1 modulators: Addressing selectivity and resistance through molecular insights - A medicinal chemistry perspective, *Bioorg. Chem.* 167 (2025 Dec) 109239, <https://doi.org/10.1016/j.bioorg.2025.109239>.
- [104] C. Peifer, D.R. Alessi, Small-molecule inhibitors of PDK1, *ChemMedChem* 3 (12) (2008) 1810–1838.
- [105] C. Dangelmaier, B.K. Manne, E. Liverani, J. Jin, P. Bray, S.P. Kunapuli, PDK1 selectively phosphorylates Thr (308) on Akt and contributes to human platelet functional responses, *Thromb. Haemost.* 112 (03) (2014) 508–517.
- [106] T. Yu, Z. Wang, W. Jie, X. Fu, B. Li, H. Xu, Y. Liu, M. Li, E. Kim, Y. Yang, The kinase inhibitor BX795 suppresses the inflammatory response via multiple kinases, *Biochem. Pharmacol.* 174 (2020) 113797.
- [107] K. Clark, L. Plater, M. Pegg, P. Cohen, Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and I κ B kinase ϵ : a distinct upstream kinase mediates Ser-172 phosphorylation and activation, *J. Biol. Chem.* 284 (21) (2009) 14136–14146.
- [108] J. Zhu, J.-W. Huang, P.-H. Tseng, Y.-T. Yang, J. Fowble, C.-W. Shiau, Y.-J. Shaw, S.K. Kulp, C.-S. Chen, From the cyclooxygenase-2 inhibitor celecoxib to a novel class of 3-phosphoinositide-dependent protein kinase-1 inhibitors, *Cancer Res.* 64 (12) (2004) 4309–4318.
- [109] L. Booth, B. Shuch, T. Albers, J.L. Roberts, M. Tavallai, S. Proniuk, A. Zukowski, D. Wang, C.-S. Chen, D. Bottaro, Multi-kinase inhibitors can associate with heat shock proteins through their NH2-termini by which they suppress chaperone function, *Oncotarget* 7 (11) (2016) 12975.
- [110] R.M. Biondi, A. Kieloch, R.A. Currie, M. Deak, D.R. Alessi, The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB, *EMBO J.* 20 (16) (2001) 4380–4390.
- [111] S.A. Moses, M.A. Ali, S. Zuohe, L. Du-Cuny, L.L. Zhou, R. Lemos, N. Ihle, A. G. Skillman, S. Zhang, E.A. Mash, In vitro and in vivo activity of novel small-molecule inhibitors targeting the pleckstrin homology domain of protein kinase B/AKT, *Cancer Res.* 69 (12) (2009) 5073–5081.
- [112] S. Hansen, J. Enquist, J. Iwig, M.E. Binnerts, G. Jamieson, J.A. Fox, A.R. Craig, PDK1 inhibitors SNS-229 and SNS-510 cause pathway modulation, apoptosis and tumor regression in hematologic cancer models in addition to solid tumors, *Mol. Cancer Ther.* 14 (12 Suppl 2) (2015), <https://doi.org/10.1158/1535-7163.TARG-15-C198>, Abstract C198.
- [113] E. Barile, S.K. De, M. Pellicchia, PDK1 inhibitors, *Pharmaceut. Patent Anal.* 1 (2) (2012) 145–163.
- [114] A. Deng, R. Fan, J. Gou, R. Sang, R. Lin, T. Zhao, J. Zhuang, Y. Hai, J. Sun, G. Wei, Innovative PDK1-Degrading PROTACs Transform Cancer Aerobic Glycolysis and Induce Immunogenic Cell Death in Breast Cancer, *Exploration (Beijing)* 5 (4) (2025 May 7) e20240031, <https://doi.org/10.1002/EXP.20240031>.
- [115] W. Liu, P. Li, Y. Mei, Discovery of SBF1 as an allosteric inhibitor targeting the PIF-pocket of 3-phosphoinositide-dependent protein kinase-1, *J. Mol. Model.* 25 (7) (2019) 187.
- [116] A. Gopalsamy, M. Shi, D.H. Boschelli, R. Williamson, A. Olland, Y. Hu, G. Krishnamurthy, X. Han, K. Arndt, B. Guo, Discovery of dibenzo [c, f][2, 7] naphthyridines as potent and selective 3-phosphoinositide-dependent kinase-1 inhibitors, *J. Med. Chem.* 50 (23) (2007) 5547–5549.
- [117] A. Emmanouilidi, C.A. Fyffe, R. Ferro, C.E. Edling, E. Capone, S. Sestito, S. Rapposelli, R. Lattanzio, S. Iacobelli, G. Sala, Preclinical validation of 3-phosphoinositide-dependent protein kinase 1 inhibition in pancreatic cancer, *J. Exp. Clin. Cancer Res.* 38 (1) (2019) 191.
- [118] C. Yang, X. Huang, H. Liu, F. Xiao, J. Wei, L. You, W. Qian, PDK1 inhibitor GSK2334470 exerts antitumor activity in multiple myeloma and forms a novel multitargeted combination with dual mTORC1/C2 inhibitor PP242, *Oncotarget* 8 (24) (2017) 39185.
- [119] D. Han, W. Wang, J.H. Jeon, T. Shen, X. Huang, P. Yi, B. Dong, F. Yang, Cooperative activation of PDK1 and AKT by MAPK4 enhances cancer growth and resistance to therapy, *PLoS Biol.* 21 (8) (2023) e3002227.
- [120] C. Liu, X. Mu, X. Wang, C. Zhang, L. Zhang, B. Yu, G. Sun, Ponatinib inhibits proliferation and induces apoptosis of liver cancer cells, but its efficacy is compromised by its activation on PDK1/Akt/mTOR signaling, *Molecules* 24 (7) (2019) 1363.
- [121] D. Komander, G.S. Kular, J. Bain, M. Elliott, D.R. Alessi, D.M. Van Aalten, Structural basis for UCN-01 (7-hydroxystaurosporine) specificity and PDK1 (3-phosphoinositide-dependent protein kinase-1) inhibition, *Biochem. J.* 375 (2) (2003) 255–262.
- [122] S. Sato, N. Fujita, T. Tsuruo, Interference with PDK1-Akt survival signaling pathway by UCN-01 (7-hydroxystaurosporine), *Oncogene* 21 (11) (2002) 1727–1738.
- [123] R. Fang, Q. Cui, J. Sun, X. Duan, X. Ma, W. Wang, B. Cheng, Y. Liu, Y. Hou, G. Bai, PDK 1/Akt/PDE 4D axis identified as a target for asthma remedy synergistic with β 2 AR agonists by a natural agent arctigenin, *Allergy* 70 (12) (2015) 1622–1632.
- [124] S.B. Kondapaka, M. Zarnowski, D.R. Yver, E.A. Sausville, S.W. Cushman, 7-hydroxystaurosporine (UCN-01) inhibition of Akt Thr308 but not Ser473 phosphorylation: a basis for decreased insulin-stimulated glucose transport, *Clin. Cancer Res.* 10 (21) (2004) 7192–7198.
- [125] J. Kortmanský, M.A. Shah, A. Kaubisch, A. Weyerbacher, S. Yi, W. Tong, R. Sowers, M. Gonen, E. O'reilly, N. Kemeny, Phase I trial of the cyclin-dependent kinase inhibitor and protein kinase C inhibitor 7-hydroxystaurosporine in combination with fluorouracil in patients with advanced solid tumors, *J. Clin. Oncol.* 23 (9) (2005) 1875–1884.
- [126] Q. Deng, X. Yu, L. Xiao, Z. Hu, X. Luo, Y. Tao, L. Yang, X. Liu, H. Chen, Z. Ding, Neolbaconal induces energy depletion and multiple cell death in cancer cells by targeting PDK1-PI3K/Akt signaling pathway, *Cell Death Dis.* 4 (9) (2013) e804-e804.
- [127] X. Luo, X. Yu, S. Liu, Q. Deng, X. Liu, S. Peng, H. Li, J. Liu, Y. Cao, The role of targeting kinase activity by natural products in cancer chemoprevention and chemotherapy, *Oncol. Rep.* 34 (2) (2015) 547–554.
- [128] R. Duan, F. Tian, J. Sun, Structural basis and energy landscape of apigenin-induced cancer cell apoptosis mechanism in PI3K/Akt pathway, *Mol. Simul.* 42 (2) (2016) 138–148.
- [129] V.M. Jansen, N.E. Bhola, J.A. Bauer, L. Formisano, K.-M. Lee, K.E. Hutchinson, A. K. Witkiewicz, P.D. Moore, M.V. Estrada, V. Sánchez, Kinome-wide RNA interference screen reveals a role for PDK1 in acquired resistance to CDK4/6 inhibition in ER-positive breast cancer, *Cancer Res.* 77 (9) (2017) 2488–2499.
- [130] V. Jansen, N. Bhola, J. Bauer, L. Formisano, P. Moore, J. Koch, C. Arteaga, Abstract PD2-06: inhibition of 3-phosphoinositide dependent protein kinase 1 (PDK1) synergizes with CDK4/6 inhibitors against ER-positive breast cancer, *Cancer Res.* 76 (4 Supplement) (2016). PD2-06-PD2-06.
- [131] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, *Science* 307 (5712) (2005) 1098–1101.
- [132] S.P.E. Jayaweera, S.P. Wanigasinghe Kanakanamge, D. Rajalingam, G.N. Silva, Carfilzomib: a promising proteasome inhibitor for the treatment of relapsed and refractory multiple myeloma, *Front. Oncol.* 11 (2021) 740796.
- [133] S. Pai, V.K. Yadav, K.-T. Kuo, N.W. Pikatan, C.-S. Lin, M.-H. Chien, W.-H. Lee, M. Hsiao, S.-C. Chiu, C.-T. Yeh, PDK1 inhibitor BX795 improves cisplatin and radio-efcacy in oral squamous cell carcinoma by downregulating the PDK1/CD47/Akt-mediated glycolysis signaling pathway, *Int. J. Mol. Sci.* 22 (21) (2021) 11492.
- [134] H. Köse, C. Schneeweis, P. Putze, C.T. Contreras, L. Ferreira, L. Witte, I. Deidda, F. Herzberg, S. Ebert, J. Jakubik, L. Moldaner, J. Todorovic, I. Träger, C. Zang, U. M. Demel, E. Hessmann, M. Kirchner, S. Rhein, J. Hoffmann, Z. Tatarova, M. Ghadimi, D. Saur, K. Kappert, P. Mertins, G. Schneider, U. Keller, M. Wirth, Targeting Mutual Dependence of Phosphatidylinositol-3-Kinase α/δ and Small Ubiquitin-Like Modifier Signaling in Pancreatic Cancer, *Gastroenterology* 170 (2) (2026 Feb) 315–329, <https://doi.org/10.1053/j.gastro.2025.08.018>.
- [135] V. Sambandam, M.J. Frederick, L. Shen, P. Tong, X. Rao, S. Peng, R. Singh, T. Mazumdar, C. Huang, Q. Li, PDK1 mediates NOTCH1-mutated head and neck squamous carcinoma vulnerability to therapeutic PI3K/mTOR inhibition, *Clin. Cancer Res.* 25 (11) (2019) 3329–3340.
- [136] E. Kostaras, T. Kaserer, G. Lazarro, S.F. Heuss, A. Hussain, P. Casado, A. Hayes, C. Yandim, N. Palaskas, Y. Yu, A systematic molecular and pharmacologic evaluation of AKT inhibitors reveals new insight into their biological activity, *Br. J. Cancer* 123 (4) (2020) 542.
- [137] N.E. Bhola, M.L. Freilino, S.C. Joyce, M. Sen, S.M. Thomas, A. Sahu, A. Cassell, C.-S. Chen, J.R. Grandis, Antitumor mechanisms of targeting the PDK1 pathway in head and neck cancer, *Mol. Cancer Ther.* 11 (6) (2012) 1236–1246.
- [138] H. Lu, Y. Lu, Y. Xie, S. Qiu, X. Li, Z. Fan, Rational combination with PDK1 inhibition overcomes cetuximab resistance in head and neck squamous cell carcinoma, *JCI Insight* 4 (19) (2019) e131106.
- [139] M. Elkabets, S. Vora, D. Juric, N. Morse, M. Mino-Kenudson, T. Muranen, J. Tao, A.B. Campos, J. Rodon, Y.H. Ibrahim, MTORC1 inhibition is required for sensitivity to PI3K p110 α inhibitors in PIK3CA-mutant breast cancer, *Sci. Transl. Med.* 5 (196) (2013), 196ra99-196ra99.
- [140] Q. Yang, P. Modi, T. Newcomb, C. Quéva, V. Gandhi, Idelalisib: first-in-class PI3K delta inhibitor for the treatment of chronic lymphocytic leukemia, small lymphocytic leukemia, and follicular lymphoma, *Clin. Cancer Res.* 21 (7) (2015) 1537–1542.
- [141] M.K. Siu, Y.-x. Jiang, J.-j. Wang, T.H. Leung, S.F. Ngu, A.N. Cheung, H.Y. Ngan, K. K. Chan, PDK1 promotes ovarian cancer metastasis by modulating tumor-mesothelial adhesion, invasion, and angiogenesis via α 5 β 1 integrin and JNK/IL-8 signaling, *Oncogenesis* 9 (2) (2020) 24.
- [142] Y. Chinen, J. Kuroda, Y. Shimura, H. Nagoshi, M. Kiyota, M. Yamamoto-Sugitani, S. Mizutani, N. Sakamoto, M. Ri, E. Kawata, Phosphoinositide protein kinase PDPK1 is a crucial cell signaling mediator in multiple myeloma, *Cancer Res.* 74 (24) (2014) 7418–7429.
- [143] T.B. Kimber, Y. Chen, A. Volkamer, Deep learning in virtual screening: recent applications and developments, *Int. J. Mol. Sci.* 22 (9) (2021) 4435.
- [144] W. Cai, M.-Q. Nguyen, N.A. Wilski, T.J. Purwin, M. Vernon, M. Tiago, A.E. Aplin, A genome-wide screen identifies PDPK1 as a target to enhance the efficacy of MEK1/2 inhibitors in NRAS mutant melanoma, *Cancer Res.* 82 (14) (2022) 2625–2639.
- [145] J.O. Schulze, G. Saladino, K. Busschots, S. Neimanis, E. Süß, D. Odadzic, S. Zeuzem, V. Hindie, A.K. Herbrand, M.-N. Lisa, Bidirectional allosteric communication between the ATP-binding site and the regulatory PIF pocket in PDK1 protein kinase, *Cell Chem. Biol.* 23 (10) (2016) 1193–1205.
- [146] E. Rozengurt, H.P. Soares, J. Sinnet-Smith, Suppression of feedback loops mediated by PI3K/mTOR induces multiple overactivation of compensatory pathways: an unintended consequence leading to drug resistance, *Mol. Cancer Ther.* 13 (11) (2014) 2477–2488.
- [147] D.P. Bondeson, C.M. Crews, Targeted protein degradation by small molecules, *Annu. Rev. Pharmacol. Toxicol.* 57 (2017) 107–123.
- [148] M. Békés, D.R. Langley, C.M. Crews, PROTAC targeted protein degraders: the past is prologue, *Nat. Rev. Drug Discov.* 21 (3) (2022) 181–200.