Perifosine: Update on a Novel Akt Inhibitor

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Current Oncology Reports 2009, **11:**102–110
Current Medicine Group LLC ISSN 1523-3790

The PI3K/Akt/mTOR pathway is aberrantly active in most human cancers and contributes to cell growth, proliferation, and survival. Akt is a nodal regulator of cellular survival pathways and an attractive target in cancer therapy. Many inhibitors of Akt are being developed. Perifosine is an oral Akt inhibitor currently being tested in phase 2 clinical trials. Unlike most kinase inhibitors, which target the adenosine triphosphate–binding region, perifosine targets the pleckstrin homology domain of Akt, thereby preventing its translocation to the plasma membrane. Single-agent activity with perifosine has been observed in sarcoma and Waldenström macroglobulinemia patients. However, the disappointing response rates of common solid tumors to perifosine as a single agent have diminished expectations and prompted further investigation into its mechanism of action. Perifosine exerts Akt-dependent and Akt-independent effects, and although many preclinical studies have documented Akt inhibition by perifosine, clinical validation of these findings is lacking. In this article, we review the clinical history of perifosine and discuss its many biologic activities.

Introduction

The phosphoinositide-3 kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is an important signal transduction pathway that controls processes integral in cancer development, including protein translation, growth, metabolism, and survival. The PI3K/Akt/mTOR pathway is frequently activated in tumors and promotes therapeutic resistance, providing a strong rationale to target it in cancer therapy. Validation of this approach came with recognition that the mTOR inhibitor temsirolimus prolonged survival in renal cell cancer patients, which led to its US Food and Drug Administration approval. However, a possible mechanism of resistance to mTOR inhibitors is feedback activation of Akt, highlighting the need to develop agents that target other pathway components.

Akt is a serine/threonine kinase that lies upstream of mTOR in the pathway because Akt phosphorylates TSC2, which de-represses Rheb to interact with FKBP38 and allow mTOR activation [1]. Yet Akt also can be downstream of mTOR because the TORC2 complex consisting of mTOR and rictor (rapamycin-insensitive companion of mTOR) can phosphorylate Akt. Aberrant Akt activation may occur through overexpression or mutation of receptor tyrosine kinases, activation of oncogenes such as Ras, inactivation of the tumor suppressor PTEN (phosphatidylinositol phosphate 3'-phosphatase) through epigenetic silencing or mutations, activating mutations or amplification of isoforms of PI3K, or mutations in the pleckstrin homology (PH) domain or genomic amplification of Akt itself. Akt is activated following activation of class I PI3K, which generates $PI(3,4)P_2$ (PIP_2) and $PI(3,4,5)P_3$ (PIP_3) (Fig. 1A). Once synthesized, PIP_2 and PIP_3 interact with the PH domain of Akt and cause its translocation to the plasma membrane, where it is phosphorylated on T308 by PDK1 and on S473 by the rictor/mTOR complex, as well as possibly by other proteins. Phosphorylation of both T308 and S473 is required for full kinase activity. Once phosphorylated, Akt dissociates from the plasma membrane and moves to various cellular compartments, where it can phosphorylate downstream substrates such as TSC2, FOXO, p21, p27, GSK3β, BAD, XIAP, and MDM2.

Several Akt inhibitors have entered early-phase clinical trials. Triciribine is a nucleoside analogue that causes dephosphorylation of active Akt. Before its characterization as an Akt inhibitor, triciribine was evaluated as a cancer agent in multiple phase 1 and 2 trials. These trials showed that triciribine had modest activity and unexpected toxicity. Based on its newly recognized activity against Akt, it is in phase 1 trials focusing on Akt inhibition as well as traditional phase 1 end points. GSK690693 is an adenosine triphosphate (ATP)-competitive Akt inhibitor also in phase 1 clinical trials. XL418 is an agent that targets both Akt and p70S6K; however, after it began phase 1 testing, the

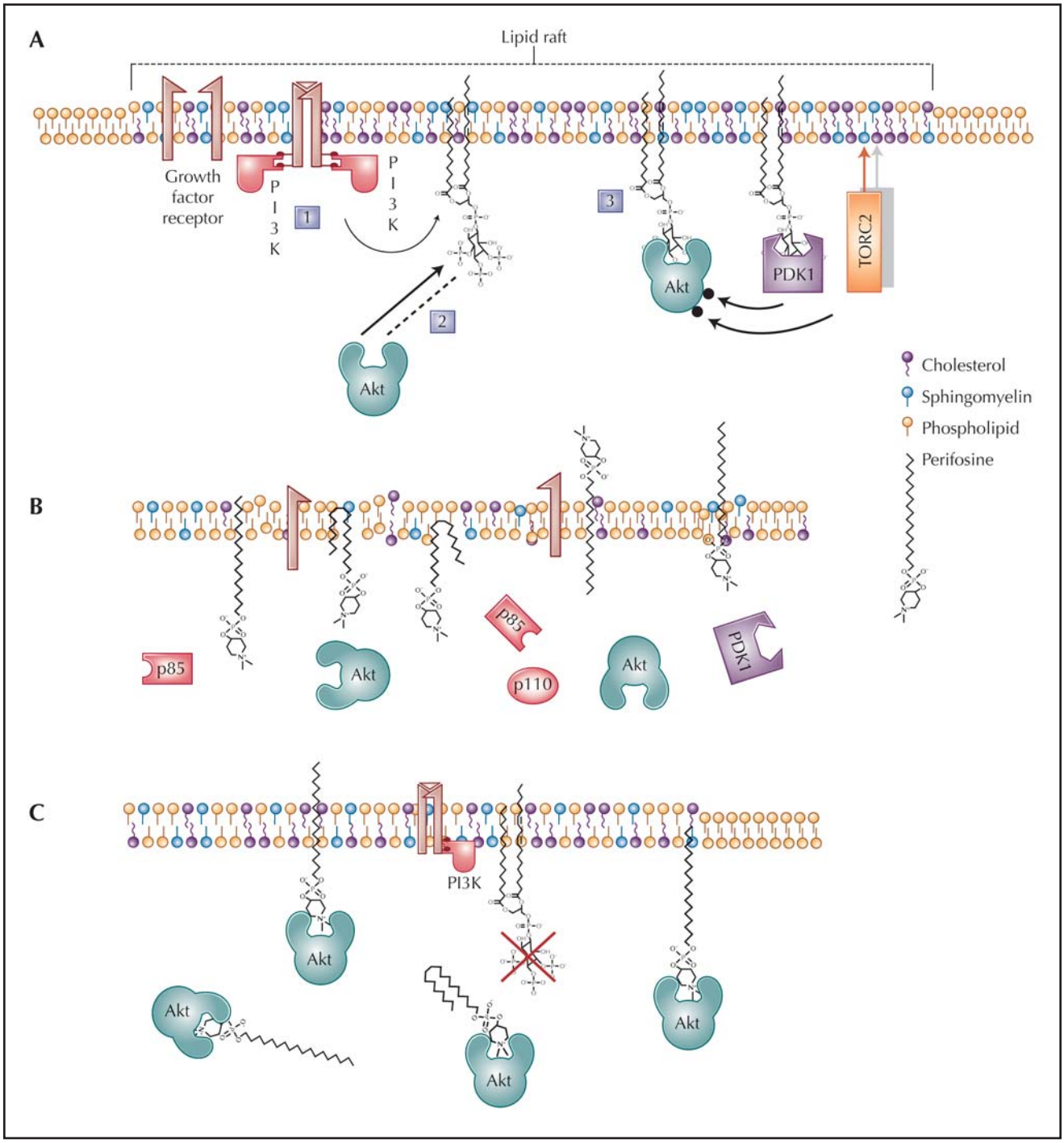

Figure 1. Events leading to Akt activation and potential inhibitory mechanism(s) of perifosine. **A,** Akt activation in lipid rafts. 1) Growth factor receptor activation via ligand binding recruits phosphoinositide-3 kinase (PI3K), which converts PI(4)P to PI(3,4)P_2 and PI(4,5)P_2 to PI(3,4,5)P_3. 2) The pleckstrin homology (PH) domain of Akt binds to PI(3,4)P_2 or PI(3,4,5)P_3 (depicted here) at the membrane. 3) Akt is activated at the membrane via phosphorylation at T308 by PDK1 and at S473 by the TORC2 complex. **B and C,** Possible mechanisms of action of perifosine. **B,** Perifosine disrupts the structure of and signaling within lipid rafts, preventing Akt recruitment to the membrane. **C,** Perifosine binds directly to and inhibits the PH domain of Akt.

trial was suspended because of low drug exposure. In addition, there are other ATP-competitive and allosteric Akt inhibitors in preclinical development [2]. It is too early to know which strategies might be successful, but specificity remains a challenge when targeting the ubiquitous ATP-binding pocket of kinases. A potentially more selective and novel approach is to target the lipid-binding PH domain of Akt, which is essential for its translocation and activation. This is the rationale behind the development of perifosine and other lipid-based Akt inhibitors [2].

Table 1. Clinical trials with perifosine as a single agent

Study	Phase	Tumor type	Patients, *n*	Response(s)	Clinicaltrials.gov identifier
Van Ummersen et al. [22]	1	Solid tumors	42	1 PR, 3 SD	
Monga et al. [25]	1	Mixed	27	4 SD	
Crul et al. [23]	1	Solid tumors	22	None	
Henderson et al. [24]	1	NSCLC	20	1 PR, 2 SD	
Hedley et al. [38]	2	Pancreatic	19	2 SD	
Ghobrial et al. [31]	2	WM	37	1 PR, 10 MR, 18 SD	
Chee et al. [35]	2	Prostate	25	None	
Marsh et al. [37]	2	Pancreatic	10	1 SD	
Bailey et al. [28]	2	Sarcoma	23	1 PR	
Knowling et al. [27]	2	Sarcoma	16	4 SD	
Argiris et al. [40]	2	Head and neck	19	1 SD	
Leighl et al. [36]	2	Breast	18	19% SD	
Ernst et al. [39]	2	Melanoma	18	3 SD	
Posadas et al. [34]	2	Prostate	19	None	
Steinert et al. [30]	2	Sarcoma	59	6 PR, 13 SD	
Stephenson et al. [26]	2	Mixed	241	3 PR, 3 SD	
Campos et al. [33]	2	HCC	13	1 PR, 5 SD	
Ph II Trial of Perifosine for Recurrent/ Progressive Malignant Gliomas	2	Glioma		Not yet reported	NCT00590954
Trial of 2 Schedules of Perifosine for Pts With Solid Tumors or Lymphomas	2	Mixed		Not yet reported	NCT00389077
Ph II Study of Perifosine for Pts With Carcinoma of the Kidney	2	Renal		Not yet reported	NCT00498966
Ph II Study of Perifosine in Pts With Refractory and Relapsed Leukemia	2	Leukemia		Not yet reported	NCT00391560

HCC—hepatocellular carcinoma; MR—minimal response; NSCLC—non–small cell lung cancer; PR—partial response; SD—stable disease; WM—Waldenström's macroglobulinemia.

History of Development of Perifosine

Perifosine is a synthetic oral alkylphospholipid (APL) with a piperidine head group. It belongs to a class of antitumor APLs that also includes edelfosine and miltefosine, and it was originally based on the structure of lysophosphatidylcholine. APLs were shown to have selectivity for neoplastic versus normal hematologic cells in vitro. In vivo, perifosine induced thrombocytosis and leukocytosis and increased myelopoiesis in murine marrow and spleen, whereas it caused apoptosis in myeloma xenografts [3]. The reason for this selectivity is unknown. In vitro, perifosine inhibits Akt at low micromolar concentrations [4–11,12••,13–21], and inhibition has been measured in many xenograft models in vivo [5,12••,14,18,21]. Thus far, the clinical results with perifosine have been modest and have not lived up to the hopes for an orally available Akt inhibitor (Table 1). However, few clinical trials with perifosine have evaluated Akt as a biomarker in patient tissues, leaving the question as to whether perifosine acts as an Akt inhibitor in humans or whether Akt inhibition is unimportant for growth of human cancers.

Clinical Summary

In preclinical studies, a schedule using a loading dose of perifosine followed by successive maintenance doses improved efficacy and toxicity and thus was adopted for clinical trials, in which the maximum tolerated dose was determined to be 150 mg orally in four loading doses and 100 mg/d for maintenance [22]. The maximum tolerated daily dose of perifosine is 200 mg/kg [23]. Unlike standard chemotherapies, perifosine induces limited bone marrow suppression. The major toxicities are gastrointestinal effects and fatigue. In phase 1 trials, partial responses were noted in one patient with sarcoma [22] and one patient with non–small cell lung cancer (NSCLC) [24]; stable disease was noted in three patients with prostate cancer [25], three patients with renal cell carcinoma

[26], one patient with liver cancer, and one patient with ocular melanoma [23].

Sarcoma was one of the tumor types selected for phase 2 evaluation because of several durable responses noted during phase 1 trials [27]. However, in two phase 2 trials of perifosine in patients with mixed sarcoma subtypes, perifosine treatment led to a partial response in 1 of 23 patients with 9% progression-free at 6 months in one study [28], and no objective responses but stable disease in 4 of 19 patients in the other [27]. These results did not meet the criteria for either trial (ie, a minimum of 40% of patients progression-free at 6 months [28], or at least one objective response and no more than 11 early progressions [27], respectively). Subsequently, an analysis of 121 sarcoma patients culled from multiple phase 1 and 2 studies showed a clinical benefit rate for perifosine of 50% [29]. A current phase 2 trial limited to chondrosarcoma and alveolar soft tissue sarcoma has reported promising activity—of 59 patients, 6 had a partial response and 13 had stable disease [30]. In addition to sarcoma, perifosine has shown promising activity in Waldenström's macroglobulinemia (WM) [31], with milder activity observed in multiple myeloma [32] and in a small set of hepatocellular carcinoma patients identified within a larger phase 2 trial [33]. However, a larger number of phase 2 trials of perifosine in prostate cancer [34,35], breast cancer [36], pancreatic cancer [37,38], melanoma [39], and head and neck cancer [40] have been disappointing (Table 1).

Despite the number of completed trials, the potential of perifosine as an Akt inhibitor in humans remains virtually unexplored. Only two studies measured Akt inhibition in patient tissues, both of which used levels of phosphorylated S473 as a surrogate for Akt activation. In a phase 2 trial of head and neck cancer, tumor biomarkers were measured by immunohistochemistry at baseline and in two patients before and after perifosine treatment [40]. In this unselected group, 64% of patient tumors had moderate or strong staining for S473 phosphorylation at baseline, and no decreases were noted in the post-perifosine biopsies. In a second study, perifosine reduced ex vivo clonogenic activity of $CD34^+$ cells from patients with acute myelogenous leukemia (AML) displaying constitutive Akt activation, but not the clonogenic activity of $CD34^+$ cells from AML patients without Akt activation [15].

Important Preclinical Studies Related to Akt

The cumulative preclinical evidence that perifosine targets Akt is convincing. Perifosine was first reported as an Akt inhibitor in 2003 [7,16]. It decreases Akt phosphorylation at S473 and T308, the two sites required for maximum kinase activity. Perifosine treatment followed by immunoprecipitation of Akt from PC3 cells decreased kinase activity in parallel with Akt dephosphorylation measured by immunoblotting [7]. In addition to downregulating endogenous active Akt, perifosine inhibits serum and growth factor–stimulated Akt, as well as insulin-stimulated movement of Akt to the plasma membrane. Akt inhibition is reversible since incubation with perifosine followed by washing and addition of fresh media led to recovery of phospho-Akt (P-Akt) [7]. The target of perifosine is the PH domain of Akt, based on its phospholipid structure. Overexpression of myristoylated Akt that is constitutively active (which bypasses the requirement for PIP_3 binding to activate Akt) negates the effect of perifosine on Akt phosphorylation, whereas transfection of WT Akt does not [7]. Likewise, overexpression of constitutively active PI3K (CA-PI3K-p110α), but not WT, overcomes perifosine-mediated inhibition of P-Akt [7].

How is perifosine able to interfere with the PH domain? Preliminary data published in abstract form suggest that perifosine inhibits binding of the Akt PH domain to artificial membranes containing 3% PIP_2 (50% inhibitory concentration [IC_{50}] > 10 μM) [41]. In contrast, it did not inhibit binding of two other PH domain–containing proteins to membranes containing phosphoinositides [DAPP1 PH domain binding to PIP_2 or PLC-δ-1 PH domain binding to $PI(4,5)P_2$ (IC_{50} > 100 μM)]. These findings suggest that perifosine selectively inhibits the PH domain of Akt, but the authors note that the binding of perifosine to the PH domain occurs with relatively low affinity and that direct binding by titration calorimetry could not be detected.

Akt inhibition by perifosine has been documented in vivo against myeloma [5], WM [18], prostate cancer [12••], and glioma [14,21] xenografts in immune-compromised mice. The most comprehensive analysis of Akt inhibition in vivo was performed by Hennessy et al. [12••], who administered perifosine to tumor-bearing mice on different schedules. In PC3 and Du145 xenografts, there was significant correlation among the cumulative dose of perifosine, tumor growth inhibition, and decreases in phosphorylation of Akt and other pathway components in the tumors [12••]. In A431 and BT474 xenografts, perifosine was ineffective in inhibiting tumor growth and did not inhibit Akt. Thus, the ability of perifosine to inhibit Akt in tumors correlated with growth inhibition in vivo. To determine the pharmacodynamics of Akt inhibition, PC3 xenografts were treated with perifosine daily and tumors were harvested on days 1, 2, 7, and 21. Significant inhibition of Akt phosphorylation at S473 was observed 7 and 21 days after perifosine administration, which led the authors to recommend assessment of Akt phosphorylation as a biomarker 7 days after perifosine administration begins [12••]. In addition, cell lines with activating PI3K/Akt genomic alterations (in PI3K, PTEN, and epidermal growth factor receptor [EGFR] overexpression) were more sensitive to perifosine in vitro than those without [12••].

Other components of the pathway

In addition to decreasing P-Akt, perifosine decreases phosphorylation of many downstream components, including P-p70S6K [12••,13], P-S6 [12••], P-4EBP-1 [13], P-GSKα/β [5,6,12••], P-FKHRL1 [5,6], and P-S112 Bad [15]. Several reports suggest that perifosine does not seem to target upstream components of the PI3K/Akt/mTOR pathway, because it did not inhibit purified PI3K [7], and did not alter the levels of P-PDK1 [5,15], mTOR, raptor (regulatory associated protein of mTOR), or rictor [15]. However, the existence of a few reports showing that perifosine does affect upstream components suggests that Akt inhibition also may be indirect. For example, perifosine can inhibit insulin-stimulated PI3K activity in intact cells [16] and decrease PTEN and total Akt levels in NSCLC cells [6]. In addition, perifosine can increase phosphorylation of growth factor receptors that stimulate Akt, such as EGFR [12••]. The mechanism underlying these effects is unclear, but some may be attributable to disturbances in membrane structure or function.

Perifosine and lipid rafts

APLs target cell membranes [42], and the long hydrocarbon chain of perifosine suggests it can easily insert into phospholipid bilayers. Perifosine has a long half-life of 137 hours and is stable in vivo [43]; thus, it may accumulate and disturb regions of the membrane involved in signaling (Fig. 1B). Consistent with this, van der Luit et al. [44] showed that perifosine may be taken up through, and accumulate in, lipid rafts. Lipid rafts are cholesterol- and sphingolipid-rich microdomains that serve as scaffolds for signal transduction. These are likely important for Akt activation, because Akt has been isolated within raft fractions (Fig. 1A) [45]. Alterations in raft composition by perifosine have been shown by Gajate and Mollinedo [46•], who demonstrated that perifosine induces clustering of DR4, DR5, FAS/CD95, FADD, and caspase 8 in leukemic raft fractions. Another way perifosine might disturb rafts is through increases in ceramide. Perifosine has been reported to elevate ceramide [47], a cytotoxic sphingolipid that also has the ability to downregulate Akt. In agreement with this finding, downregulation of sphingomyelin synthase I (SMSI) abrogates the toxicity of perifosine [44], and cells that were made resistant to edelfosine or perifosine [48•] had decreased levels of SMSI [49]. Because ceramide can be produced from sphingomyelin by the action of sphingomyelinases, this suggests that sphingolipid signaling could be important for the cytotoxicity and Akt inhibition associated with perifosine.

Cytotoxic mechanism of perifosine

In addition to targeting Akt, perifosine possesses other biologic activities that may contribute to its cytotoxicity. These include activation of p38 and JNK (7), inhibition of phosphatidylcholine synthesis [44], increased expression of p21 [50] and DR5 [6,46•,51], inhibition of migration and adhesion [18], downregulation of MEK and ERK phosphorylation [15], and decreased expression of P-glycoprotein [4], survivin, and β-catenin [10].

Perifosine may induce cytostatic [9,13,50,52] or cytotoxic responses. In hematologic malignancies, perifosine primarily causes apoptosis through activation of the extrinsic pathway via stimulation of death receptors that increase cleavage of caspases 3, 8, and 9. In these cells, critical mediators of perifosine-induced apoptosis include CD95/FAS [4,15,46•], lipid rafts [4,44,46•], and JNK [5,15], but not Akt [5]. In epithelial cancer cells, apoptosis [6,8,16], cell cycle arrest [9,13,50], or necrosis may result [9]. In NSCLC cells, perifosine caused apoptosis accompanied by an increase in DR5, a decrease in c-FLIP, and caspase 8 and 9 cleavage. Apoptosis was inhibited by expression of constitutively active Akt, as well as by small interfering RNA knockdown of caspase 8 and DR5 [6]. Thus, multiple mechanisms of action likely contribute to the anticancer effects of perifosine.

Combination strategies with perifosine

The concept of "oncogene addiction" asserts that some cancers depend on one gene or a few genes for their survival and maintenance of the malignant phenotype. Although PI3K/Akt/mTOR pathway activation is required for the development and/or maintenance of certain stages of cancer, tumor heterogeneity and clinical experience suggest that combination therapy will be required to achieve remission or cure in most patients. In vitro, perifosine has been combined successfully with UCN-01 [53], histone deacetylase (HDAC) inhibitors [47], etoposide [11], TRAIL (tumor necrosis factor–related apoptosis-inducing ligand) [6,51], erlotinib [8], cetuximab [17], 17-DMAG [20], dexamethasone, doxorubicin, melphalan, and bortezomib [5]. In vitro, death by the combination of perifosine and HDAC inhibitors was shown to depend on Akt inhibition, ERK activation, and reactive oxygen and ceramide production [47]. With the combination of perifosine and TRAIL, death depended on DR4 and DR5 expression, but not Akt [51]. In mice bearing human tumors in vivo, combinations of perifosine and temozolomide [13] or radiation [54] have shown promise. In AML blasts, perifosine increased sensitivity to etoposide [15].

These preclinical data, combined with the observation that Akt inhibition increases the responsiveness to chemotherapy and radiation, have prompted several phase 1 combination studies with perifosine (Table 2). These include evaluation of perifosine combined with radiation [55], lenalidomide [56], sorafenib [57], sunitinib [58], docetaxel [59], paclitaxel [60,61], imatinib, or gemcitabine [62]. The most promising combination results have been in myeloma. In a phase 2 trial in patients with relapsed/refractory multiple myeloma, perifosine and dexamethasone achieved a partial or minimal response (MR) in

Table 2. Clinical trials with perifosine in combination

Study	Phase	Tumor type	Combination	Patients, *n*	Response(s)	Clinicaltrials.gov indentifier
Schreeder et al. [57]	1	Solid tumors	Sorafenib	20	7 SD	
Allerton et al. [58]	1	Solid tumors	Sunitinib	14	1 PR, 3 SD	
Jakubowiak et al. [56]	1	Myeloma	Lenalidomide + dexamethasone	12	1 NCR, 1 VGPR, 3 PR, 1 MR, 1 SD	
Goggins et al. [60]	1	Solid tumors	Paclitaxel every 3 wk	12	2 SD	
Ebrahimi et al. [61]	1	Solid tumors	Paclitaxel every wk	12	5 SD	
Weiss et al. [62]	1	Solid tumors	Gemcitabine	22	4 PR, 6 SD	
Cervera et al. [59]	1	Solid tumors	Docetaxel + prednisone	39	None	
UCN-01 and Perifosine in Treating Pts With Relapsed or Refractory Leukemia, CML, or High Risk Myelodysplastic Syndromes	1	Leukemia	UCN-01		Not yet reported	NCT00301938
Vink et al. [55]	1	Solid tumors	Radiation	21	None	
Richardson et al. [32]	2	Myeloma	Dexamethasone	48	1 MR, 22 SD (single agent)	
				31	4 PR, 8 MR, 15 SD (combo)	
Richardson et al. [63]	2	Myeloma	Bortezomib ± dexamethasone	15	2 PR, 1 MR, 3 SD (bortezomib)	
					2 MR, 3 SD (bortezomib + dexamethasone)	
Ph II Study of Perifosine Plus Gleevec for Pts With GIST	2	GIST	Imatinib		Not yet reported	NCT00455559
Randomized Placebo-Controlled Study of Perifosine in Combo With Single Agent Chemo	2	Mixed	Chemotherapy		Not yet reported	NCT00398879

CML—chronic myelogenous leukemia; GIST—gastrointestinal stromal tumor; MR—minimal response; NCR—near complete response; PR—partial response; SD—stable disease; VGPR—very good partial response.

38% and stable disease in 47% [32]. In a phase 1 trial of perifosine and bortezomib with or without dexamethasone, 20% of patients had a partial response or MR and 20% had stable disease with perifosine plus bortezomib, whereas 13% had an MR and 20% had stable disease with perifosine plus bortezomib and dexamethasone [63]. These combinations have advanced into a phase 2 trial. Given the promising activity observed in patients with myeloma and the accessibility of tumor cells in this malignancy, future trials with perifosine in multiple myeloma should analyze biomarkers to help determine the mechanism of action of perifosine in humans.

Conclusions

Although there is strong evidence that perifosine inhibits Akt in preclinical models of cancer, perifosine possesses Akt-independent activities as well. Death receptors, lipid rafts, and JNK also are important in the death caused by perifosine, depending on the cell type. In vitro, perifosine interferes with the normal function of the PH domain of Akt. Yet it remains unclear whether perifosine does so by disrupting membrane microdomains crucial to Akt activation (Fig. 1B) or whether it binds directly to the PH domain of Akt, thereby displacing the natural PIP_2 and PIP_3 ligands (Fig. 1C). Regardless, the most promising clinical results with perifosine have been in sarcoma and WM patients, but almost none of the reported trials attempted to measure Akt inhibition. Given the overall modest clinical activity observed with perifosine, it is crucial to develop reliable assays that measure Akt and to employ them in clinical trials with Akt inhibitors. If perifosine inhibits Akt and the clinical response is poor, this would cast doubt on existing assumptions about the value of Akt as a target in cancer. If Akt is not inhibited, this should prompt examination of Akt-independent mechanism(s) of action or pharmacodynamic properties of perifosine, to better guide the clinical development of this unusual agent.

Disclosures

No potential conflicts of interest relevant to this article were reported.

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