Review

Cancer Research

AKT as a Therapeutic Target for Cancer

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Abstract

Many cellular processes in cancer are attributed to kinase signaling networks. V-akt murine thymoma viral oncogene homolog (AKT) plays a major role in the PI3K/AKT signaling pathways. AKT is activated by PI3K or phosphoinositide-dependent kinases (PDK) as well as growth factors, inflammation, and DNA damage. Signal transduction occurs through downstream effectors such as mTOR, glycogen synthase kinase 3 beta (GSK3β), or forkhead box protein O1 (FOXO1). The abnormal overexpression or activation of AKT has been observed in many cancers, including ovarian, lung, and pancreatic cancers, and is associated with increased cancer cell proliferation and survival. Therefore, targeting AKT could provide an important approach for cancer prevention and therapy. In this review, we discuss the rationale for targeting AKT and also provide details regarding synthetic and natural AKT-targeting compounds and their associated studies.

Introduction

The AKT serine/threonine kinase, also known as protein kinase B (PKB), is an oncogenic protein that regulates cell survival, proliferation, growth, apoptosis, and glycogen metabolism (Fig. 1; ref. 1). AKT is activated by phosphorylation on Thr308 or Ser473 and it phosphorylates a variety of downstream protein substrates, including GSK3β, Bcl-2-associated death promoter, forkhead in rhabdomyosarcoma, and mouse double minute 2 homolog (2). Phosphorylated AKT (pAKT) has been implicated in the deregulation of apoptosis, proliferation, and cell motility because of its induction of signals that interfere with normal regulatory mechanisms activating mTOR (3, 4). Overexpression of pAKT is considered to be a therapeutic target for treating malignant tumors. For example, phosphorylation of AKT at Ser473 has been reported to promote breast cancer metastasis (5). At least one clinical study suggests that 20%–26% of patients with breast cancer expressing high levels of pAKT (Ser473) appear to be sensitive to treatment with paclitaxel or adjuvant doxorubicin plus cyclophosphamide and showed improved overall survival (OS) or disease-free survival (DFS; ref. 6). Therefore, the purpose of this review is to consider and analyze the role of AKT signaling in carcinogenesis and to examine progress in creating effective inhibitors of this kinase to treat various cancers.

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AKT as a Target in Cancer Therapy

AKT is overactivated in cancer

The overactivation of AKT is a common molecular characteristic of human malignancies (7, 8). Expression of certain oncogenes or loss of particular tumor suppressor genes can result in activation of the PI3K/AKT signaling pathway. For example, the amplification of Erb-B2 receptor tyrosine kinase 2 (ErbB2), mutations of EGFR/PI3K, or the loss of the tumor suppressor protein, PTEN, as well as mutations or amplification of AKT itself can result in increased AKT signaling in tumor cells (9). Mutated or deleted PTEN is common in many tumors and leads to overactivation of the PI3K/AKT network. Restoration of PTEN function enhances $p21^{WAF1/CIP1}$-regulated cell-cycle inhibition by blocking PI3K/AKT signaling (10). Inhibition of NFκB-driven-COX-2 expression by cis-9,trans-11–conjugated linoleic acid contributed to antitumor effects by decreasing inhibitor of nuclear factor kappa kinase (IKK) activity and blocking PI3K/AKT signaling in TPA-treated hairless mouse skin *in vivo* (11). Ginsenoside Rg3 was also reported to attenuate NFκB signaling, possibly through the inactivation of AKT and ERKs and destabilization of mutant p53, leading to apoptosis of MDA-MB-231 breast cancer cells (12). Increased AKT1 activity has been observed in approximately 40% of breast and ovarian cancers and in over 50% of prostate cancers, and overactivation of AKT2 has been observed in about 30%–40% of ovarian and pancreatic cancers (13, 14). Increased AKT3 activity has been observed in estrogen receptor (ER)- or androgen receptor (AR)-deficient breast or prostate cancer cells, respectively, suggesting that AKT3 may contribute to the aggressiveness of hormone-independent cancers (15, 16). High expression levels of the phosphorylated AKT protein were observed in 75 of 83 (90.4%) cases of esophageal squamous cell carcinoma (ESCC) compared with normal esophageal mucosa (27.7% or 23/83 cases; ref. 17). This could affect the function of other cancer-associated proteins and lead to drug resistance. AKT-overexpressing cells displayed resistance to cisplatin, which was associated with overexpression of the antiapoptotic Bcl-xL protein that delayed the activation of the p53 signaling pathway (18, 19).

Overexpression or amplification of AKT1 and AKT2 is also associated with acquired resistance of ovarian cancer cells to paclitaxel (20). Constitutive pAKT (Ser473) expression under starvation conditions was exhibited by 13 of 19 lung cancer cell

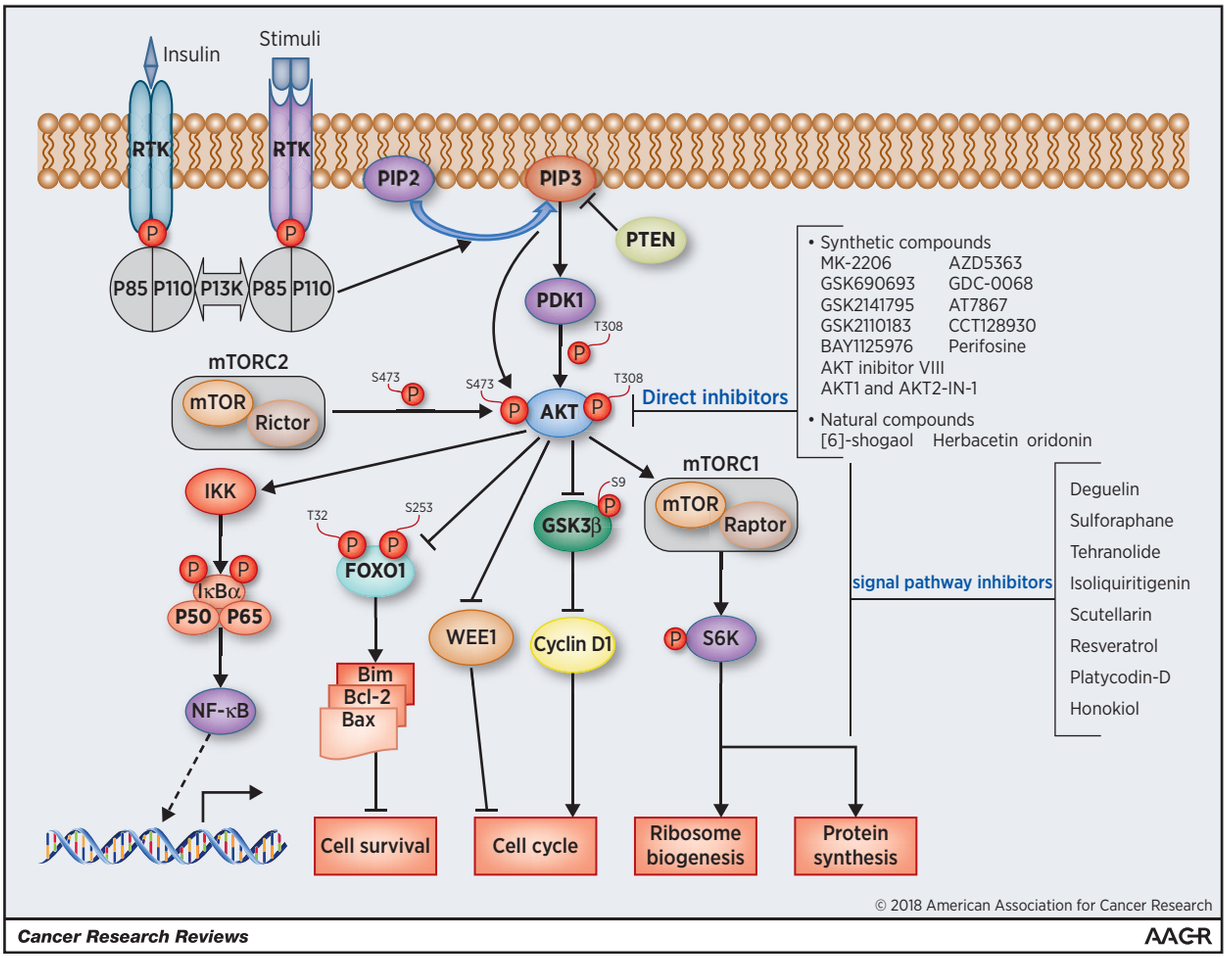

Figure 1.

Schematic diagram of the PI3K/AKT signaling pathway. Upstream target proteins of AKT are stimuli-induced receptor tyrosine kinases (RTK) and include PI3K, PDK, and mTOR complex 2 (mTORC2). Activated AKT phosphorylates downstream target proteins, including FOXO1, WEE1, GSK3β, and mTORC1, and the signaling results in cancer cell survival, cell-cycle effects, ribosome biogenesis, or protein synthesis. Activation of AKT can be inhibited by downregulation of upstream targets. Upstream target mediators include deguelin, sulforaphane, tehranolide, isoliquiritigenin, scutellarin, resveratrol, platycodin-D, or honokiol. Direct synthetic AKT inhibitors are MK-2206, AZD5363, GSK690693, GDC-0068, GSK2141795, GSK2110183, AT7867, CCT128930, BAY1125976, perifosine, AKT inhibitor VIII, and AKT1 and AKT-IN-1 and natural inhibitors, [6]-shogaol, herbacetin, and oridonin.

lines (21). The activation appeared to be due to increased upstream signaling. In an examination of lung cancer tissues, the percentage of AKT-positive samples in cancer and adjacent tissues was 76.47% (39/51) and 38.46% (5/13), respectively. Also, a significant correlation was observed between AKT expression and grade of cancer tissue differentiation ($P < 0.05$; ref. 22). In gastric cancer, cytoplasmic AKT expression was markedly increased in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)-mutant tumors (23). The AKT rs1130233 polymorphism is associated with increased phosphorylated AKT expression in *H. pylori*-positive individuals. Also the polymorphism and *H. pylori* infection showed a significant interaction in the progression from normal tissue to atrophic gastritis and gastric cancers in human males (24). Phosphorylated AKT (Ser473) was also highly expressed in human papillomavirus (HPV) -related oropharyngeal squamous cell carcinoma in contrast to pAKT on Thr308. Notably, pAKT (Ser473) expression increased in primary tumors to progressive nodal disease (21.1%; $P < 0.011$; ref. 25).

pAKT as a prognostic marker in the clinic

The expression of pAKT is negatively correlated with survival in patients with ESCC ($r = -0.473$; $P < 0.01$) and the cumulative survival rate of pAKT-positive patients was significantly lower than that of pAKT-negative patients ($P < 0.01$; ref. 17). In patients with non–small cell lung cancer (NSCLC), pAKT (Ser473) levels were elevated in patients with acquired EGFR tyrosine kinase inhibitor (TKI) resistance (26). Moreover, the OS of pAKT-negative patients was 34.5 months, which is double the OS of 15.2 months of pAKT-positive patients ($P = 0.0015$). The progression-free survival (PFS) rates for patients undergoing EGFR-TKI treatment was 14.5 months for pAKT-negative patients compared with 6.1 months for pAKT-positive patients ($P = 0.0037$; ref. 26). These data revealed a potentially important role for increased pAKT levels as a novel biomarker for predicting a reduced initial EGFR-TKI response in patients (26). Activation of AKT also predicted the development of diffuse intrinsic pontine gliomas based on the loss of PTEN (27). Nuclear pAKT (Ser473)

was predominantly overexpressed in 371 of 522 diffuse large B-cell lymphoma (DLBCL) cases. Compared with patients with low levels of pAKT, those with high expression had relatively poor PFS ($P = 0.0027$) and OS ($P = 0.047$). The 5-year PFS of patients with DLBCL with high expression of pAKT was 45.8% compared with 61% of patients with low expression (HR = 1.54; ref. 28).

Synopsis of Current AKT Inhibitors

Identifying AKT inhibitors that can block PI3K/AKT signaling by directly inhibiting AKT kinase activity or pAKT expression could attenuate cancer growth. Kinase inhibitors comprise three types (29). The first type competes with ATP and forms hydrogen bonds with the hinge region of the kinase, the second kind binds partially in the ATP-binding site and extends to the gatekeeping area and an adjacent allosteric site, and the third type functions as an allosteric inhibitor. Most AKT inhibitors in clinical development inhibit AKT 1, 2, and 3 and are referred to as pan-AKT inhibitors. Inhibitors comprise both synthetic and naturally occurring compounds (Fig. 1; Tables 1 and 2).

Synthetic compounds

GSK690693 (aminofurazan class) is an ATP-competitive pan-AKT kinase inhibitor [IC_{50}, 2 nmol/L (AKT1), 13 nmol/L (AKT2), and 9 nmol/L (AKT3); ref. 9]. It suppresses the downstream AKT target, GSK3β, and reportedly inhibits the growth of BT-474 breast cancer cells in a xenograft mouse model (30). Clinical trials (NCT00493818, NCT00666081, phase I) testing GSK690693 have been cancelled or closed because the drug induced severe hyperglycemia (31).

GSK2141795 (uprosertib) is another ATP-competitive pan-AKT inhibitor. This drug was reported to enhance cisplatin-induced apoptosis *in vitro* and decrease phosphorylation of proline-rich AKT substrates in an SKOV3 ovarian cancer xenograft mouse model (32, 33). GSK2141795 was tested in a phase II clinical trial in combination with the MAPK/ERK1/2 inhibitor, trametinib, in patients with advanced melanoma ($n = 48$). The results showed that the combination had an acceptable toxicity profile, but unfortunately, patients expressing either the wild-type neuroblastoma *RAS* viral oncogene homolog *(NRAS)* or mutant *NRAS* (34) did not respond to the treatment.

GSK2110183 (afuresertib) is an orally available ATP-competitive and pan-AKT kinase inhibitor. It attenuated phosphorylation levels of various AKT substrates (e.g., GSK3β, PRAS40, FOXO, and caspase-9) in BT-474 breast cancer and LNCaP prostate cancer cell lines expressing $ERBB2^{+}$, PIK3CA, K111N, or PTEN null (35). Dumble and colleagues reported that GSK2110183 showed a 65 and 21% effectiveness against hematological and solid tumor cells, respectively (35). An open-label, phase II single institution trial of a combination of intravenous infusion of ofatumumab and oral GSK2110183 in relapsed or refractory chronic lymphocytic leukemia patients (NCT01532700) was closed in June 2017. Results have been submitted to ClinicalTrials.gov, but are not yet publicly available.

AZD5363, another oral ATP-competitive pan-AKT inhibitor (IC_{50} < 10 nmol/L), showed a favorable pharmacokinetic and toxicity profile in a BT474c breast cancer xenograft mouse model (36). AZD5363 monotherapy suppressed proliferation of 41 of 182 solid/hematologic tumor cell lines with an IC_{50} < 3 μmol/L. Oral administration of AZD5363 resulted in the reduction of PRAS40, GSK3β, and S6 phosphorylation in a BT474c xenograft mouse model (37). Chronic oral treatment with AZD5363 inhibited growth of trastuzumab-resistant human epidermal growth factor receptor 2 ($HER2^{+}$) breast cancer cells in a xenograft model. Furthermore, AZD5363 also significantly enhanced the anticancer activity of docetaxel, trastuzumab, or lapatinib in breast cancer xenograft mouse models (37). One clinical trial (NCT01353781) was conducted with AZD5363 in patients with solid tumors harboring an AKT mutation ($AKT1^{E17K}$), including ER-positive/triple-negative breast, gynecologic, lung, prostate, and colorectal cancers. The median PFS among the patients with heavily pretreated $AKT1^{E17K}$-mutant tumors was 5.5 months in patients with ER-positive breast cancer, 6.6 months in patients with gynecologic cancer, and 4.2 months in patients with other solid tumors. These results suggest that AZD5363 might be effective against tumors harboring the $AKT1^{E17K}$ mutation (38).

GDC-0068 (ipatasertib) is an ATP-competitive pan-AKT inhibitor exerting antiproliferative and antisurvival effects against several cancer cell lines by inhibiting the PI3-K/AKT pathway (39). Inhibition of AKT activity by this compound blocked cell-cycle progression and reduced viability of cancer cell lines, including PC-3 (PTEN deletion) prostate cancer cells, BT474M1 (PIK3CAK111N mutant and HER2-amplified) breast cancer cells, and IGROV-1 ($PTEN^{T319fsX1/Y155C}$ and $PIK3CA^{*1069W}$) ovarian cancer cells (40). GDC-0068 inhibited AKT signaling, not only in cultured human cancer cell lines (PC-3, BT474M1, IGROV-1), but oral administration decreased ovarian, prostate, breast, glioblastoma, colorectal, NSCLC, and melanoma xenograft cell growth in which AKT expression was elevated (40). GDC-0068 also effectively inhibited growth *in vivo* in tumors expressing AKT activated by genetic alterations, including PTEN loss, PIK3CA mutations/amplifications, or HER2 overexpression (40). Furthermore, a combination of GDC-0068 and docetaxel or carboplatin attenuated xenograft growth of PC-3 (prostate), MCF7-neo/HER2 (breast), OVCAR3 (ovarian) cancer cells in mice. A first-in-man phase I study of ipatasertib (NCT01090960) demonstrated robust and safe targeting of AKT in patients with solid tumors and indicated that this drug was well tolerated and inhibited PRAS40, GSK3β, and mTOR in paired on-treatment biopsies (41). Ipatasertib is now being further evaluated in phase II studies.

AT7867 is an ATP-competitive inhibitor of AKT1/2/3 and ribosomal protein S6 kinase beta-1 (p70S6K)/protein kinase A (PKA) that exhibits little activity outside the protein kinase A, G, and C (AGC) kinase family. AT7867 significantly inhibited the growth of PTEN-null U87MG human glioblastoma cell xenografts and its bioavailability in mice was 44% when administered orally (42).

CCT128930 is an ATP-competitive and selective AKT2 inhibitor ($IC_{50} = 6$ nmol/L) and has a 28-fold selectivity for AKT2 compared with the closely related PKA kinase. CCT128930 exhibited antitumor activity against PTEN-null U87MG glioblastoma and HER2-positive, PIK3CA-mutant BT474 breast cancer xenografts in mice (43). Neither AT7867 nor CCT128930 has been enrolled in clinical trials at this time.

MK-2206 is an orally available allosteric AKT1/2 inhibitor and exhibits IC_{50} values of 8, 12, and 65 nmol/L against AKT1, 2, and 3, respectively (44). Allosteric AKT inhibitors do not result in hyperphosphorylation of AKT at Ser473/Thr308, unlike ATP-

Table 1. The efficacy of inhibitors in animal experiments

Compound name	Cancer	Animal model	Concentration	Efficacy	Reference
Synthetic inhibitors					
GSK690693	Ovarian, prostate, and breast cancer	Human SKOV-3, LNCaP, BT-474, or HCC-1954 cancer cells in xenograft mouse model	10, 20, or 30 mg/kg administered by i.p. (once daily for 21 days)	Maximal inhibition of 58 to 75% was observed at the end of dosing period with the 30 mg/kg/day dose	9
GSK2141795	Epithelial ovarian cancer	SKOV3 cell subcutaneous xenografts in NU/NU-Foxn-1 mice	30 mg/kg for 1–72 hours, prior to [18F] FDG administration (∼8 MBq) through a jugular vein cannula	30 mg/kg GSK2141795 reduced [18F] FDG signal reaching a maximum of 68%	32
GSK2110183	Breast cancer	SCID mice bearing BT474 tumor xenografts, nude mice bearing SKOV3, CAPAN-2, or HPAC cancer cell xenografts	100 mg/kg by oral gavage daily	No experiment on tumor growth	35
AZD5363	Breast and renal cancers	BT474c, HCC-1954, or 786-0 cells implanted into SCID or nude mice	75–200 mg/kg by oral administration twice daily	100 mg/kg AZD5363 resulted in 80% inhibition ($P < 0.0001$, $HER2^+$-amplified, PIK3CA-mutant BT474c xenografts). 150 mg/kg AZD5363 caused pronounced tumor regression (129% inhibition; $P < 0.0001$, HCC-1954 breast cancer xenograft) and resulted in partial regression (125% inhibition; $P < 0.0001$, 786-0 PTEN-null renal cancer xenografts)	37
GDC-0068	Prostate cancer	PC3 cells inoculated subcutaneously to nude mice	0–100 mg/kg qd or bid by oral treatment	100 mg/kg GDC-0068 qd reached maximum tumor growth inhibition (79%, $P < 0.0001$); 50 mg/kg GDC-0068 bid resulted in a nearly equivalent tumor growth inhibition. qd dosing of 100 mg/kg	39
	Ovarian cancer	TOV-21G.x1 (PTEN-null, $PIK3CA^{H1047R}$, $KRAS^{G13C}$ ovarian cancer cell line) in xenograft (nude) mouse model	0–100 mg/kg daily by oral gavage	GDC-0068 elicited tumor stasis at 25 mg/kg in the TOV21G.x1 model, with partial regression observed at 50 mg/kg or higher	40
AT7867	Uterine sarcoma and glioblastoma	Human MES-SA uterine sarcoma cells or U87MG human glioblastoma cells in xenograft (nude) mouse model	20 mg/kg AT7867 i.p. or 90 mg/kg AT7867 p.o. once every 3 days	37% and 38% decreases on tumor volume at 20 mg/kg i.p or 90 mg/kg p.o., respectively, on MES-SA tumor xenografts; 51% decrease of U87MG cell xenografts at 20 mg/kg i.p. dose	42
CCT128930	Glioblastoma and breast cancer	PTEN-null U87MG human glioblastoma and BT474 cells in xenograft (nude) model	50 mg/kg CCT128930 i.p. daily for 5 days (U87MG xenografts) or 40 mg/kg CCT128930 i.p. twice daily for 5 days (BT474 xenografts).	48% and 29% decreases on T/C ratio in U87MG xenografts and BT474 xenografts, respectively.	43
MK-2206	Breast cancer and ovarian cancer	SK-OV-3 and HCC70 cells in xenograft (nude) mouse model	MK-2206 120 mg/kg, orally, 3 x a week for 2 weeks, or 360 mg/kg, orally, once a week for 2 weeks	MK-2206 alone showed moderate efficacy ($P < 0.0001$); the combination with lapatinib yielded a significantly greater inhibition of HCC70 or SK-OV-3 xenograft tumor growth ($P < 0.0001$).	44

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Table 1. The efficacy of inhibitors in animal experiments (Cont'd)

Compound name	Cancer	Animal model	Concentration	Efficacy	Reference
BAY 1125976	Breast cancer and prostate cancer	KPL-4 and MCF-7 breast cancer cells and AKTE17K mutation carrying LAPC-4 prostate cancer xenograft (nude) mouse model	25 or 50 mg/kg BAY 1125976 oral administration	Daily oral treatment with 25 or 50 mg/kg BAY 1125976 with T/C volume ratios of 0.14 and 0.08 in KPL-4 xenograft mouse model; with T/C volume values of 0.25 and 0.25 ($P < 0.001$) in MCF-7 xenograft mouse model; with T/C volume values of 0.32 and 0.27 (both $P < 0.001$) in prostate PDX cancer model	50
	Colon cancer	HCT116, T84, DLD-1, and HCT15 cancer cell xenograft (nude) mouse models	100 mg/kg once daily for 5 consecutive days each week in combination with PD0325901 (5 mg/kg) by oral gavage	BAY 1125976 single dose had only marginal or modest antitumor effects, however, AKTi in combination with PD0325901 synergistically suppressed tumor growth in all 4 models	51
AKT1 and AKT2-IN-1	Ovarian cancer	A2780 cancer cell xenograft (nude) mouse model	50 mg/kg compound was dosed subcutaneously 3x per day twice a week for 5 cycles	A statistically significant decrease in mean tumor weight as compared to the vehicle control was noted for mice in the treatment group.	52
Perifosine	Gliomas	DF1 cell xenograft ion Neonatal Ef-luc Ntv-a mice	Oral administration of 30 mg/kg perifosine, or a combination of perifosine and temozolomide for 3 to 5 days	Ki-67 staining index was significantly reduced in the perifosine group (Ki-67 staining index $= 3.2 \pm 1.1\%$, $n = 3$, $P = 0.0010$) compared with control. The combination group (temozolomide + perifosine) had the lowest Ki-67 staining index ($1.7 \pm 1.2\%$, $n = 3$, $P = 0.0005$).	53
Natural inhibitors					
[6]-Shogaol	Non–small cell lung cancer	NCI-H1650 cell xenograft (nude) mouse model	10 or 40 mg/kg intraperitoneal 3x a week for 3 consecutive weeks.	Tumor volume decreased 30.2% and 64.2% in the 10 and 40 mg/kg group, respectively; Ki-67 staining showed 56.2% and 93.8% at 10 mg/kg and 40 mg/kg, respectively.	67
	Skin carcinogenesis	TPA-induced tumor promotion in mouse epidermis in female ICR mice	2.5 μmol [6]-Shogaol dissolved in 200 μL acetone smeared on the skin	Pretreatment with curcumin, 6-gingerol, or 6-shogaol reduced the number of tumors per mouse by 39.4, 70.6, and 91.2% at 2.5 μmol dose and the percentage of animals with tumors was decreased by 18, 28, and 58%, respectively, compared to control group	69
Oridonin	Esophageal squamous cell carcinoma	Patient-derived xenograft mouse (SCID) model	40 or 160 mg/kg for oral administration	160 mg/kg of oridonin significantly reduced tumor growth compared to vehicle group with almost a 50% decrease in Ki-67 staining	70

(Continued on the following page)

Table 1. The efficacy of inhibitors in animal experiments (Cont'd)

Compound name	Cancer	Animal model	Concentration	Efficacy	Reference
Herbacetin	Cutaneous squamous cell carcinoma and melanoma	Solar–UV induced-skin tumor mouse model and SK-MEL-5 cell xenograft (nude) mouse model	100 or 500 nmol for UV-induced skin carcinogenesis; 0.2 or 1 mg/kg herbacetin injection 3 × per week	Herbacetin treatment significantly decreased the number and volume of skin papillomas relative to the TPA-only-treated group ($P < 0.05$); it also decreased the volume of melanoma growth relative to the vehicle-treated group ($P < 0.05$)	71
Tehranolide	Breast cancer	Spontaneous mouse mammary tumor (SMMT) developed in female BALB/c mice	5.64 μg/mouse/day by intraperitoneal injection	The apoptosis index of the positive group in the tehranolide-treated group was significantly higher than control group ($P < 0.01$) by TUNEL staining	79
Isoliquiritigenin	Adenoid cystic carcinoma	ACC-M cell xenografts in female BALB/c nude mice	0.5 or 1 g/kg orally for 30 consecutive days.	The tumor volume showed around 57% and 85% decreases in 0.5 and 1 g/kg-treated group compared to vehicle-treated group.	81
	Breast cancer	MDA-MB-231 cell xenografts in nude mice	50 or 100 mg/kg by i.p. injection	Isoliquiritigenin led to significant inhibition of tumor growth with decreases of 43.1% and 62.3% in tumor weight at 50 and 100 mg/kg, respectively, compared with control group; TUNEL positive nuclei in tumor tissues from mice treated with isoliquiritigenin (50 or 100 mg/kg) increased to 3.3 and 9.2 fold, respectively.	82
	Breast cancer	MMTV-PyMT transgenic mouse model	50 mg/kg/d by oral administration	50 mg/kg treatment showed significant decreases of tumor weight and tumor size compared to control group ($P < 0.01$)	83
Scutellarin	Hepatocellular carcinoma	SK-Hep1 cell orthotopic liver xenograft model in nude mice	50 mg/kg/d by i.p. injection	The numbers of lung and intrahepatic metastatic tumors in the scutellarin-treated group were significantly less than in the controls ($P < 0.05$)	85
Honokiol	Glioma	U87 MG cell orthotopic brain tumor model in nude mice	20 mg/kg/d by i.p. 2 × per week for 2 weeks	The expression of LC3 in tumor tissues showed a 2.5-fold increase after honokiol administration compared to vehicle	96

Abbreviations: p.o., orally; qd, once daily; bid, twice daily.

competitive inhibitors (45). MK-2206 exhibited potent antiproliferative activity against various cancer cell types harboring PI3KCA mutations, PTEN loss, upstream RTK gene amplification or overactivation, and mutation of AKT itself. A dose-escalation clinical trial investigated tolerability, safety, and MTD of MK-2206 in 33 patients with advanced solid tumors and in 72 patients previously treated with carboplatin/paclitaxel, docetaxel, or erlotinib (NCT00848718; refs. 46, 47). A phase II trial (NCT01333475) investigated the combination of MK-2206 with selumetinib, a MAPK kinase 1/2 (MEK1/2, MAP2K1/2) inhibitor (48). Unfortunately, no objective responses were observed. However, determining whether the lack of response was due to a suboptimal dose and schedule or to an AKT/MEK-independent activation of ERKs was difficult. A recent clinical trial (NCT01369849) examined the efficacy of combining MK-2206 with bendamustine and rituximab in relapsed or refractory chronic lymphocytic leukemia. The overall response rate was a promising 92% and the median PFS and treatment-free survival was 16 and 24 months, respectively. These results indicate that AKT

Table 2. Clinical trials of AKT inhibitors

Drug	Clinical trial number	Cancer type	Clinical phase	Combination	Dosage	Note	Year
Synthetic inhibitors							
MK-2206	NCT 00848718	Advanced solid tumors	Phase I	Carboplatin/paclitaxeldocetaxel or erlotinib	45 mg QOD or 200 mg Q3W (Arm 1); MAD 200 mg Q3W (Arm 2) and 135 mg QW (Arm 3)	Combination study was well-tolerated in cytotoxic and targeted therapies	2012
	NCT 01333475	Advanced colorectal cancer	Phase II	Selumetinib	135 mg orally once weekly and AZD6244 hydrogen sulfate 100 mg orally once daily administered in 28-day cycles	The desired level of target inhibition was not achieved	2015
	NCT 01369849	Relapsed or refracttory chronic lymphocytic leukemia	Phase II	Bendamustine and rituximab	Orally on days 1, 8, 15, and 22, rituximab i.v. on day 1, bendamustine hydrochloride i.v. over 30-60 minutes on days 1–2. Treatment repeats every 28 days	AKT inhibition combined with chemoimmunotherapy is a promising novel treatment combination in CLL	2017
AZD5363	NCT 01353781	Advanced solid tumor	Phase I	No	Capsules administered orally as a single dose, and then multiple twice-daily dosing following 3- to 7-day washout	AKT1E17K may be therapeutically targeted by AZD5363 in patients with diverse tumor types	2015
GDC-0068	NCT 01090960	Advanced solid tumor	Phase I	No	Oral repeating dose Range from 25 to 125 mg/m^2/day for 28 days per cycle	Ipatasertib was well tolerated and multiple targets (i.e., PRAS40, GSK3β, and mTOR) were inhibited in paired on-treatment biopsies	2017
Perifosine	NCT 00776867	Recurrent/refracttory pediatric CNS and solid tumors	Phase I	No	Ranging from 25 to 125 mg/m^2/day for 28 days per cycle	Perifosine was safe and feasible in patients with recurrent/refractory pediatric CNS and solid tumors	2017
	NCT 00019656	Relapsed and refractory lymphoproliferative diseases	Phase II	Rorafenib	50 mg twice daily for 1 month and combination therapy, perifosine plus sorafenib, 400 mg twice daily	Perifosine and sorafenib combination therapy is feasible with manageable toxicity and demonstrates promising activity in patients with Hodgkin lymphoma	2014
	NCT 00398879	Metastatic colorectal cancer	Phase II	Capecitabine	P-CAP50 mg orally once daily, days 1 to 21 and CAP, 825 mg/m^2 orally twice daily (days 1 to 14 or CAP, 825 mg/m^2 orally twice daily, days 1 to 14) in 21-day cycles until disease progression	P-CAP showed promising clinical activity compared with CAP in previously treated patients with mCRC	2011
	NCT 01049841	Recurrent pediatric solid tumor	Phase I	Temsirolimus (mTOR inhibitor)	Perifosine (25–75 mg/m^2/day) and temsirolimus (25–75 mg/m^2, IV weekly)	The combination of AKT and mTOR inhibitors was safe and feasible in patients	2017

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Table 2. Clinical trials of AKT inhibitors (Cont'd)

Drug	Clinical trial number	Cancer type	Clinical phase	Combination	Dosage	Note	Year
	NCT 00776867	Resistant neuroblastoma	Phase I/Ib	No	Perifosine 50-75 mg/m^2/day after a loading dose of 100–200 mg/m^2 on day 1	Perifosine monotherapy was confirmed to be a safe and well-tolerated treatment in children with HR-NB despite the role of AKT in normal tissues	2017
	NCT 00448721	Renal cell carcinoma	Phase II	No	100 mg daily	Perifosine demonstrated activity in patients with advanced RCC after failure on VEGF-targeted therapy, but not superior to currently available second-line agents	2012
	NCT 00058214	Biochemically recurrent prostate cancer	Phase II	No	900 mg orally on day 1, then 100 mg daily starting 24 hours later	Modest single-agent clinical activity but did not meet prespecified PSA criteria even though well tolerated	2007
Natural inhibitors							
Resveratrol or grape powder	NCT 00256334	Colon cancer	Phase I		20–160 mg/day for resveratrol 125 mg/day for grape extract	Resveratrol modulated Wnt signaling *in vivo* in colon cancer and normal colonic mucosa	2005
	NCT 01370889	Healthy, no evidence of disease	Phase I		1 gm resveratrol once daily for 12 weeks orally	Daily 1 gm dose of resveratrol has favorable effects on estrogen metabolism and sex steroid hormone binding globulin among overweight and obese postmenopausal women	2011
Ginger root extract	NCT 01344538	Colorectal cancer	Phase II		Ginger root extract (pure encapsulations) 2.0 g per day (10:1 extract)	Ginger reduced proliferation in normal-appearing colorectal epithelium and increased apoptosis but did not alter 15-PGDH protein expression	2007
Sulforaphane	NCT 01228084	Recurrent prostate cancer (Adenocarcinoma)	Phase II		Capsules include 200 μmol (total daily) sulforaphane orally from week 1 day 1 to week 20 day 7	No statistical analysis provided for proportion of patients who achieved a 50% decline in prostate-specific antigen (PSA) levels	2010

Abbreviations: MAD, maximum administrated dose; mCRC, metastatic colorectal cancer; Q3W, once every 3 weeks; QOD, once every other day; QW, once weekly.

inhibition combined with chemo-immunotherapy might be a promising treatment option (49).

BAY 1125976 is another AKT1/2 inhibitor that binds into an allosteric binding pocket formed by the kinase and PH domains of AKT1 or AKT2 (50). BAY 1125976 was well-tolerated *in vivo* and demonstrated dose-dependent antitumor efficacy in multiple tumor models with an activated PI3K/AKT/mTOR pathway, including AKT (E17K) mutant- or PTEN loss–driven tumors (50). BAY 1125976 effectively blocked AKT signaling by inhibiting pAKT and its downstream target, eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), in AKT-mediated tumorigenesis (51). A phase I dose escalation study (NCT01915576) for "all comer" patients was completed in 2016.

"AKT1 and AKT2-IN-1" is an allosteric inhibitor of AKT1 (IC_{50}, 3.5 nmol/L) and AKT2 (IC_{50}, 42 nmol/L). AKT1 and AKT2-IN-1 is dependent on the PH-domain for AKT inhibition and is selective for AKT1/2 rather than AKT3 (IC_{50} = 1.9 μmol/L), or other members of the AGC kinase family (>50 μmol/L). AKT1 and AKT2-IN-1 was well tolerated in mice and attenuated levels of AKT in blood samples. AKT1 and AKT2-IN-1 treatment in an A2780 ovarian carcinoma cell xenograft model showed 80% and 75% inhibition of AKT1 and AKT2, respectively (52).

Perifosine is an oral alkyl-phospholipid AKT inhibitor that blocks the translocation of AKT to the plasma membrane and its subsequent phosphorylation, thereby exerting a marked cytotoxic effect against human tumor cell lines (53). An *in vivo* study showed that perifosine combined with temozolomide was more effective than temozolomide treatment alone against platelet-derived growth factor B-driven gliomas in mice (53). Patel and colleagues reported that perifosine (20 μmol/L) induced cell-cycle arrest at both the G_1 and G_2–M phases and increased $p21^{WAF1}$ expression in both tumor suppressor p53 wild-type and knockout cells (54). In contrast, perifosine had no effect on cyclin-dependent kinase inhibitor 1A ($p21^{WAF1}$) or cell cycle in p21-knockout variants expressed in head and neck squamous carcinoma cells. In addition, perifosine markedly decreased the level of pAKT beginning at 10 minutes and lasting up to 24 hours and moderately decreased the level of pS6 from 1 to 24 hours in rats (55). This compound was demonstrated to have manageable or no toxicity from 25 to 125 mg/m^2/day in a phase I clinical study (NCT00776867) in recurrent/refractory pediatric central nervous system and solid tumor patients (56). Perifosine combined with sorafenib showed promising activity against Hodgkin lymphoma in a phase I clinical study (NCT00019656; ref. 57). This compound was also examined as a second- or third-line therapy in combination with capecitabine in patients with metastatic colorectal cancer (NCT00398879; ref. 58). Additional phase I and phase I/Ib clinical studies confirmed that perifosine monotherapy was a safe and well-tolerated treatment in children with high-risk neuroblastoma (HR-NB; ref. 59). The combination of perifosine and an mTOR inhibitor (temsirolimus) was found to be safe and reasonable at a dose level of 25–75 mg/m^2/day orally and 25–75 mg/m^2 i.v. weekly, respectively, in patients with recurrent/refractory pediatric solid tumors (NCT01049841, NCT00776867; refs. 59, 60). Although perifosine has been studied in many trials, it has not been found to be superior to other first- or second-line cancer therapies. In 2012, Cho and colleagues (61) demonstrated that perifosine was well-tolerated and showed activity in patients with advanced renal cell carcinoma (RCC) after failure on VEGF-targeted therapy. However, its activity was not superior to currently available second-line agents (NCT00448721). Apart from this, a phase II trial (2007) of perifosine (NCT00058214) in patients with biochemically recurrent, hormone-sensitive prostate cancer also showed that the response to perifosine as a single agent did not pass prespecified prostate-specific antigen (PSA) criteria. However, 20% of patients showed PSA reduction in the NCT00058214 study (62), suggesting that perifosin might have modest single-agent clinical activity. Overall, perifosine was shown in several clinical trials to have significant activity either as a single agent or in combination therapy. However, more mechanistic research is needed for its development into an effective therapeutic agent.

The AKT inhibitor, VIII, was developed based on 2, 3-diphenylquinoxaline, which was discovered through a high-throughput screening effort to identify compounds capable of inhibiting all 3 AKT isoforms (63, 64). As reported, many tumor cell lines, including HT29 (colon), MCF7 (breast), A2780 (ovarian), and LNCaP (prostate), are highly sensitive to VIII (63). This inhibitor effectively decreased cell proliferation and increased apoptosis by translocation of phosphatidylserine (PS), induction of cleaved caspase-9, caspase-3, and PARP (65).

Natural compounds

Because the structure of AKT has been solved, its many functions have been gradually revealed. Many pharmaceutical companies and academic laboratories are actively developing natural compounds that directly target AKTs. Numerous preclinical investigations have shown that some herbs and natural phytochemicals can inhibit AKT activity directly (Fig. 1).

[6]-Shogaol from ginger root inhibited the PI3K/AKT/mTOR signaling pathway by directly targeting AKT1 and AKT2, but not PI3K or mTOR. Its inhibitory activity occurred through its binding to an allosteric site of AKT at the lower interface between the N- and C-lobes of the kinase domain. This compound suppressed proliferation of NSCLC, hepatocarcinoma, skin, and ovarian cancer cells (66–69). Another compound, oridonin (*rabdosia rubescens*), decreased cell proliferation *in vitro* and patient-derived xenograft growth *in vivo* by directly targeting AKT competitive with ATP (70). Herbacetin found in flaxseed directly inhibits the kinase activities of AKT1/2 and ornithine decarboxylase (ODC), but not MEKs or ERKs. This resulted in suppressed tumor growth in DMBA/TPA or solar UV-induced skin carcinogenesis and melanoma in *in vitro* and *in vivo* models (71, 72). Deguelin (*Mondulea Sericea*) attenuated tobacco-induced lung tumorigenesis and premalignant human bronchial epithelial cell growth by downregulating the PI3K/AKT signaling pathway (73, 74). Sulforaphane (SFN) from cruciferous vegetables induces G_2–M phase arrest and apoptosis of osteosarcoma cells and, combined with 2 Gy of radiation, induced apoptosis by suppressing AKT and ERKs expression (75–77). SFN reportedly inhibits tumor cell growth because the electrophilic carbon in the isothiocyanate moiety reacts with the nucleophilic group on amino acids and covalently modifies them, thus decreasing kinase activity of PI3K, AKT, and NF-κB (78). Tehranolide (*Artemisia diffusa*) decreased growth in MCF-7 breast cancer cell xenografts in mice through the production of reactive oxygen species (ROS) and downregulation of pAKT (79). Isoliquiritigenin (ISL) from licorice root possesses anticancer activities such as inhibition of proliferation and angiogenesis, induction of cell-cycle arrest and apoptosis, and obstruction of metastasis (80, 81). ISL suppressed growth and induced apoptosis both in MCF-7 and MDA-MB-231 breast cancer cells and repressed the arachidonic acid metabolic network and

inactivated the AKT pathway *in vivo* (82). ISL increases PTEN expression by decreasing miR-374a expression, thereby inhibiting AKT signaling in breast cancer therapies (82–84). Scutellarin is an active flavonoid from *Erigeron breviscapine* and blocked the migration and invasion of HepG2 cells by inhibiting the STAT3/girdin/AKT axis (85). Resveratrol (RES) is found in grapes, berries, and peanuts and inhibited activation of multiple survival pathways, including the PI3K/AKT pathway, thereby inducing cancer cell apoptosis (86, 87). RES reportedly inhibited proliferation and migration of hepatocellular carcinoma and colon cancer cells through the downregulation of the PI3K/AKT pathway by modifying sirtuin 1-mediated posttranslational modification and elevating bone morphogenetic protein 7 (88, 89). RES induced apoptosis by specifically targeting pAKT and mediators of apoptosis in H460 lung cancer cells (90). It also influenced autophagic/apoptotic death in drug-resistant oral cancer cells mediated through adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) and AKT/mTOR signaling (91). RES was able to induce cell-cycle arrest in human gastric cancer MGC803 cells by regulating the expression of the PTEN/PI3K/AKT signaling pathway (92). Platycodin-D (PD) from *Platycodon grandiflorum* root induced autophagy of NSCLC cells by inhibiting PI3K/AKT/mTOR signaling and activating the c-Jun N-terminal kinases (JNKs)/p38 MAPK signaling pathways (93). The combination of PD and the synthetic AKT inhibitor MK-2206 attenuated the feedback activation of the AKT pathway and led to blockade of AKT/4E-BP1 function, thereby inhibiting proliferation and inducing apoptosis of NSCLC (94). Honokiol (*Magnolia officinalis*) induced autophagic cell death by downregulating PI3K/AKT/mTOR signaling in U87MG human glioma or mouse neuroblastoma cells (95, 96).

Although clinical trials have not yet been conducted with any natural compounds targeting AKT kinase activity, patients are either enrolled or are being enrolled in clinical trials focusing on colon cancer prevention (NCT00256334) or in postmenopausal women with high body mass index (NCT01370889) to test RES and grape powder extract, respectively. Other trials include ginger extract in colon cancer prevention (NCT01344538; ref. 97) and sulforaphane in recurrent prostate cancer (NCT01228084). In the NCT00256334 study, the most significant results were observed in subjects treated with grape powder (80 g per day; GP80). GP80 decreased *cyclin D* and *axinII* gene expression, which are Wnt (wingless-type murine-mammary-tumor virus integration site) target genes in colon cancer (98). Treatment with RES (1 g/day) decreased the risk of breast cancer in postmenopausal women with high BMI by increasing both urinary 2-hydroxyestrone (73%) and sex hormone-binding globulin (10%) (99). However, researchers and clinicians are still trying to identify proper biomarkers and optimize doses of natural compounds as chemopreventive or therapeutic agents for use in the clinic.

Perspective and Conclusions

For many years, AKT has been considered as an attractive target for cancer therapy and prevention. AKT inhibitors have taken a large step forward through the development of synthetic and natural compounds that directly target AKT or AKT-related signaling pathways. Thus far, only a few AKT inhibitors have been approved by the FDA for cancer treatment. Miltefosine (Impavido, phospholipid drug) originally was used against cutaneous or mucosal leishmaniasis (100). As an AKT inhibitor, miltefosine affects human immunodeficiency virus-1 (HIV-1)-infected macrophages (101) and patients are being enrolled in a clinical trial to test its effectiveness as a cancer therapeutic (NCT02366884). Because PI3K is an upstream kinase of AKT, either inhibitors of PI3K or mTOR could affect the AKT signaling cascades. The FDA-approved PI3K or mTOR inhibitors include idelalisib (a PI3K delta inhibitor) used in patients with leukemia and lymphoma; copanlisib (a PI3K alpha/delta inhibitor) to treat adult patients with relapsed follicular lymphoma; and sirolimus (an mTOR inhibitor) to treat patients with lymphangioleiomyomatosis with gene mutations of the *tuberous sclerosis complex 2* gene in renal cell carcinoma (RCC); and everolimus (an mTOR inhibitor) to treat RCC, pancreatic, and breast cancers.

Targeting AKT has therapeutic potential but also has pitfalls because of the complex signaling pathway network. MK-2206 treatment downregulated the expression level of p-AKT (both Ser473 and Thr308) in DLBCL cells but its upstream proteins, including PI3K, mTORC2, and p-FAK, were overactivated to compensate (28). The evaluation of DLBCL patient samples also indicated that Myc and Bcl-2 were also overexpressed along with upregulation of phosphorylated AKT (28). To avoid the phenomenon of compensatory resistance, AKT-specific inhibitors could be used in combination with PI3K or mTOR inhibitors or dual or triple inhibition of those targets to reach better pharmacokinetic properties. NVP-BEZ235 is well-known as a dual inhibitor of PI3K and mTOR, and synergistically with cisplatin inhibits tumor growth in FaDu hypopharyngeal squamous cell carcinoma (102). This compound is also synergistically effective with sunitinib against prostate cancer (103) and with temozolomide against glioblastoma multiforme (104). However, this compound causes dephosphorylation of AKT (Thr308) for only a short time and hyperphosphorylation occurs again with continuous exposure. INK128, an mTOR inhibitor, also caused dephosphorylation of S6K1 and AKT (Ser 473) for 1 hour, but AKT was again phosphorylated at both Ser and Thr residues again by 24 hours. Interestingly, the PDK1 inhibitor, GSK233470, also could not overcome the reoccurring phosphorylation successfully (105). Predictably, the combination of PI3-K/mTOR (NVP-BEZ235) and an AKT inhibitor (MK-2206) suppressed the hyperphosphorylation of AKT at 24 hours and also showed synergistically decreased cell viability with the combination index (CI) ranging from 0.08 to 0.87 (105). In line with the *in vitro* work, a combination of NVP-BEZ235 and MK-2206 treatment also resulted in an additional 46% reduction in tumor weight compared with single treatment with NVP-BEZ235 (105). Thus, this brings an entirely new perspective in the triple-targeting of PI3K/AKT/mTOR signaling in cancer therapy.

Multivariate survival analysis also revealed that Myc or Bcl-2 elevation and TP53 mutation status could contribute to patient survival time, thus making the overexpression of phosphorylated AKT an insignificant independent prognostic marker in DLBCL OS (28). Moreover, *PIK3CA* mutation and *PTEN* loss might affect AKT signaling and the *PIK3CA*-mutant cells such as MCF-7, HCT-116, HCT-15, and SW-948 showed strongly diminished AKT signaling. Furthermore, *PIK3CA*-mutant cells with low levels of phosphorylated AKT expression exhibited less dependence on AKT signaling although PIK3CA was still essential for tumorigenesis (106).

Mutation of AKT in exon 20 has been reported to be 100% in gastric cancer. In addition, mutation levels of 56% frequently occur in exon 9 and 40% in exon 1. But until now, these mutations

have not been reported to be statistically significant (107). An E17K mutation in the AKT1 pleckstrin homology domain (PH domain) has been identified in human colorectal, breast, and ovarian cancers functioning to activate AKT1 and its downstream signaling and subsequently stimulating tumorigenesis (108). Overall, AKT signaling might be influenced by upregulation of PI3K or mTOR in compensation for or mutation of upstream signaling. Multiple targeting of the entire AKT signaling pathway or combination therapy will be novel strategies for future cancer therapies. Therefore, AKT is a potential therapeutic focus of cancer and should continue to gain more and more attention as a target for the development of a variety of AKT inhibitors for cancer prevention and treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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