Protein Kinase C as a Therapeutic Target
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The recognition of protein kinase C (PKC) as the long sought-after receptor for the tumor-promoting phorbol esters established the potential role of PKC in carcinogenesis and as a potentially important target for cancer therapeutics (1, 2). PKC is a family of serine/threonine kinases that regulate a variety of cell functions including proliferation, gene expression, cell cycle, differentiation, cytoskeletal organization, cell migration, and apoptosis (3). The PKC family was the first recognized receptor of diacylglycerol. Aberrant PKC activation can lead to diseases of cellular dysregulation such as cancer and diabetes. The existence of multiple isoforms of PKC raised the question of whether each PKC isozyme has a specific function. The PKC family includes isozymes [α, βI, βII, γ, δ, ε, θ, η, λ/ι (mouse/human), and ζ] which are involved in signal transduction from membrane receptors to the nucleus (Fig. 1; ref. 4). Differences in PKC isozyme protein structure and substrate preferences have allowed the family to be divided into three groups (2). First, the conventional PKC isozymes (α, βI, βII, and γ) are calcium-dependent and are phospholipid- and diacylglycerol-activated kinases. Second, the novel isozymes (δ, ε, μ, and θ) are calcium-insensitive, phospholipid-dependent, and diacylglycerol-dependent. Third, the atypical PKC isozymes (η and ι/λ) are both calcium- and diacylglycerol-insensitive enzymes. The activation mechanism of the PKC isozyme family is clearly different among the three subgroups: conventional, novel, and atypical PKC, but whether or not each isozyme in a subgroup has a specific function or activation mechanism has not been clarified. The activation and degradation of the PKC isozymes is controlled spatially and temporally (5). PKC is a single polypeptide with four conserved regions and four variable regions having a COOH-terminal catalytic domain and a NH2-terminal regulatory domain (2). The multiple functions of PKC in signal transduction are regulated by targeting PKC to specific intracellular compartments. Several PKC isozymes may be capable of phosphorylating the same substrate. The constitutive, lipid-dependent protein kinase activity of purified PKC delayed the realization that PKC phosphorylation plays a fundamental role in their catalytic activities. PKC phosphorylation in vivo is well documented and is important in the maturation of the enzyme to a fully functional form localized correctly in the cell.

The identification of physiologically relevant substrates of the 11 known PKC isozymes is of obvious importance for a complete understanding of the mechanisms by which this family of serine/threonine protein kinases relays information from intracellular stimuli to biological responses. Many proteins have been identified as PKC substrates both in vitro and in vivo. Interestingly, however, knowledge of PKC substrates is not currently matched by the detailed understanding of PKC regulation (2–4, 6). PKC isoforms are widely distributed in tissues, although some isoforms are selectively expressed. PKCγ is selectively expressed in the central nervous system and spinal cord, PKCθ is expressed by skeletal muscle and hematopoietic cells, and PKCβ is expressed in pancreatic islet cells, monocytes, brain, and retinal tissue (2).

Many kinases display overlapping substrate specificities in vitro and can functionally compensate for each other in single-gene knockout experiments. Early investigations regarding the role of PKC isoforms in various intracellular signaling events were based on a linear paradigm (6). Recent studies show that signaling molecules aggregate to form multiprotein complexes, a phenomenon that seems to hold a number of various types of molecules in close proximity. The formation of these complexes may facilitate signal transduction and allow the modulation of biological functions within the cell. PKC isoforms do not function in isolation but exist in complexes with other signaling molecules.

PKCβII, PKCε and PKCγ are targets in phorbol ester-mediated tumor promotion/progression, whereas PKCδ is tumor-suppressive (Fig. 1). Disulfide forms of thiols have oxidative regulatory effects on PKC isozymes and PKC S-thiolation by disulfiram induces differential regulatory effects on PKC isozymes that correlate with the cancer-preventive activity of disulfiram (7, 8). Overexpression of PKCδ stimulates apoptosis in a wide variety of cell types through a mechanism that is incompletely understood (9). PKCδ-deficient cells are impaired in their response to DNA damage–induced apoptosis, suggesting that PKCδ is required for an apoptotic response to stress. Using adenoviral vectors, Santiago-Walker et al. (9) found a modest increase in PKCδ activity but PKCα or PKCζ activity was not able to selectively stimulate quiescent cells to initiate G(1) phase cell cycle progression. The PKCδ-infected cells arrest in S phase and proceed to caspase-dependent apoptotic cell death. Further delineating the PKC isozymes, Gustafson et al. (10) showed that atypical PKCs is required for Bcr-Abl-mediated resistance of human K562 chronic myelogenous leukemia cells to paclitaxel-induced apoptosis. When Bcr-Abl was expressed in Bcr-Abl-negative HL-60 promyelocytic leukemia cells, the expression of PKCβII, PKCζII, and PKCδ was induced whereas expression of PKCδ was decreased. Thus, Bcr-Abl-mediated transformation involves transcriptional activation of the PKCζ gene leading to Bcr-Abl-mediated chemoresistance.

Understanding the mechanisms by which PKC contribute to malignancy remains a challenge (11). One model critically implicates conventional PKCβII (diacylglycerol-responsive) and atypical PKCζ (diacylglycerol-unresponsive) in cancer cell transformation and invasion. PKCζ is markedly up-regulated in human colon and lung tumors, in an in vivo model...
of chemically induced colon carcinogenesis, and in the expression of activated PKC\(\alpha/\epsilon\)-promoted hypersensitivity of mice to a carcinogen.

**Skin Cancer**

The PKC family is important in normal keratinocyte biology and in skin diseases, especially skin cancer (12). Keratinocyte apoptosis induced by UV radiation is a major protective mechanism from photocarcinogenesis. The constitutively active catalytic domain of PKC\(\delta\) is an apoptotic effector generated by caspase-3 cleavage of full-length PKC\(\delta\) in response to a wide variety of apoptotic stimuli including UV radiation (13). The activated PKC\(\delta\) catalytic domain triggers the redistribution and activation of Bax that can directly induce cytochrome \(c\) release. PKC\(\delta\) also activates upstream components of the death effector pathway to insure apoptosis. The cleavage and activation of PKC\(\delta\) are critical components of UV-induced apoptosis in human keratinocytes. Inactivation of PKC\(\delta\) can promote the survival of keratinocytes exposed to UV radiation (14). Recently, Aziz et al. (15) showed that PKC\(\delta\) overexpression in transgenic mice failed to suppress the induction of squamous cell carcinoma developed by repeated exposure to UV radiation.

Transgenic mice that overexpress PKC\(\varepsilon\) protein in basal epidermal cells and cells of the hair follicle develop papilloma-independent metastatic squamous cell carcinoma elicited by 7,12-dimethylbenz(\(a\))anthracene-initiation and 12-O-tetradecanoylphorbol-13-acetate promotion. Chronic exposure of PKC\(\varepsilon\) transgenic mice to UV radiation indicates that PKC\(\varepsilon\) signals tumor necrosis factor-\(\alpha\) release, which is linked to photosensitivity in these mice (16). The overexpression of PKC\(\varepsilon\) in the epidermis of mice may lead to the induction of specific cytokines that perturb normal hematopoiesis in bone marrow, resulting in a granulocytic skew toward neutrophils and eosinophils (17, 18). Both the transgenic overexpression of PKC\(\varepsilon\) in mouse epidermis and human squamous cell carcinoma indicate that high expression of PKC\(\varepsilon\) in the epidermis may lead to a microenvironment

![Fig. 1. Schematic of placement of PKC isoforms in intracellular signaling pathways.](image-url)
suitable for the development of squamous cell carcinoma by a paracrine mechanism.

**Colon and Gastric Cancer**

PKC activity is higher in actively proliferating colonic epithelial cells than in their quiescent counterparts, suggesting a role for PKC in proliferation. Colonic epithelial cells express multiple PKC isozymes that are differentially modulated during colon carcinogenesis, suggesting a direct connection between PKC and colon carcinogenesis (10, 11).

PKCα expression is decreased in azoxymethane-induced rat and sporadic human colon tumors (19). Human CaCo-2 gastric cancer cells stably transfected with PKCα showed that increased expression of PKCα induced cellular differentiation and limited survival. Elevated expression of PKCβII is an early event in colon carcinogenesis. Transgenic expression of PKCβII in mouse colon led to hyperproliferation and increased susceptibility to colon carcinogenesis that correlated with decreased expression of transforming growth factor-β type II receptor. Yu et al. (20) showed that transfection with PKCβIII induced the expression of cyclooxygenase-2 in rat intestinal epithelial cells in culture and in transgenic PKCβIII mice. Transgenic mice expressing PKCβII in colonic epithelium show that high expression of PKCβII is both necessary and sufficient to confer susceptibility to azoxymethane-induced colon carcinogenesis (21). PKCβII regulated its own expression in rat intestinal epithelial cells in culture and in animals through a mitogen-activated protein/extracellular signal-regulated kinase kinase–dependent signaling pathway. In the colon epithelium, elevated expression of PKCβII seems to be procarcinogenic, acting through both cyclooxygenase-2 and transforming growth factor-β signaling pathways.
PKC\(\alpha\) is implicated as a critical downstream effector of oncogenic Ras in the colonic epithelium (11). Using the synthetic ether lipid, 1-O-hexadecyl-2-O-methyl-rac-glycerol, which inhibits PKC activation by competing with the natural PKC activator, diacylglycerol, Liou et al. (22) showed that human tumor cell lines with oncogenic p21ras mutations were much more sensitive to PKC inhibition by 1-O-hexadecyl-2-O-methyl-rac-glycerol than tumor cell lines with normal K-ras alleles. In transgenic mice expressing constitutively active PKC\(\alpha\) in the colon epithelium, PKC\(\alpha\) activity was required for oncogenic Ras- and carcinogen-mediated colon carcinogenesis. PKC isoforms may act in concert to promote malignancy in the colon because transgenic expression of PKC\(\beta\)II in mouse colon induces hyperproliferation and increased susceptibility to colon cancer through pathways that include the activation of Ras, PKC\(\alpha\)/Rac1, and Mek leading to invasion in intestinal epithelial cells (23). PKC phosphorylation promotes rapid dissociation of K-Ras from the plasma membrane and association with intracellular membranes, including the outer membrane of mitochondria, in which phosphor-K-Ras interacts with Bcl-XL (24). Interestingly, gastric adenocarcinoma SNU-1 cells respond to dietary phytoalexin resveratrol by loss of membrane-associated PKC\(\delta\) and an increase in cytosolic PKC\(\alpha\) prior to the up-regulation of antiproliferative and proapoptotic signals (25).

**Prostate Cancer**

PKC\(\alpha\) and PKC\(\delta\) promote apoptosis in androgen-dependent prostate cancer cells (26). Stewart and O’Brien (27) found 12-O-tetradecanoylphorbol-13-acetate–induced PKC\(\alpha\) activation

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**Fig. 2** Continued.
in epidermal growth factor receptor transactivation and signaling to Erk1/2 activation in PC-3 cells. The PKC-selective kinase inhibitors, GF109203X and Go6983, blocked phorbol ester–induced epidermal growth factor receptor transactivation, indicating a requirement for PKC, and supporting PKCα inhibition as a therapeutic approach for prostate cancer.

PKCε expression in LNCaP cells was sufficient to confer a significant resistance to phorbol ester induction of Bax oligomerization suggesting the association of PKCε with Bax and the neutralization of apoptotic mitochondrial death-signaling pathways (28). Failure of hormonal therapy frequently involves an outgrowth of PKCε-positive cells in recurrent prostate cancer. Wu et al. (29) found a functional interplay among the integrin receptors, PKCε and protein kinase B/Akt, in recurrent CWR-R1 prostate cancer, suggesting that sustained activation of these β1 integrins induced an androgen independent–like behavior in these cells.

Ovarian, Breast, and Endometrial Cancer

Atypical PKCα is critical in the establishment and maintenance of epithelial cell polarity and is genomically amplified and overexpressed in serous epithelial ovarian cancers (30). PKCα is markedly increased and mislocalized in all serous ovarian cancers. In nonserous ovarian cancers, increased PKCα is associated with decreased overall survival. Thus, PKCα may be an oncogene in ovarian cancer regulating epithelial cell polarity and proliferation. Compared with normal tissues, endometrial cancers also exhibit alterations in the expression profile or in the activities of the PKC family, and aberrant levels or activation of specific PKC isoforms are postulated to contribute to endometrial neoplasia and transformation. Lahn et al. (31) found that PKCα increased in breast cancers with low or negative estrogen receptor levels. In breast cancer cells, the indolocarbazole PKC inhibitor, Go6976, abrogated S and G2 cell cycle arrest (32). In p53-mutated human SKOV3 ovarian cancer xenograft tumors, the administration of Go6976 and caffeine was able to restore the response to cisplatin (33).

Lung Cancer

Atypical PKCα is highly expressed in human non–small cell lung carcinoma (NSCLC) lines, whereas the closely related PKC isozyme, PKCζ, is undetectable in these cells (34). PKCα is a critical lung cancer gene that activates a Rac1 → Pak → Mek1,2 → Erk1,2 signaling pathway required for transformed growth. PKCα may be an oncogene in NSCLC because: (a) PKCα is overexpressed in most NSCLCs, (b) PKCα expression predicts poor survival in patients with NSCLC, (c) the PKCα gene is frequently amplified in NSCLC lines and primary NSCLC, (d) gene amplification drives PKCα expression in NSCLC lines and primary tumors, and (e) PKCα disruption signaling with a dominant-negative PKCα allele blocks the transformed growth of human NSCLC cells harboring PKCα gene amplification (34, 35). PKCα is implicated in carcinogen-induced survival of malignant cells (36). Stallings-Mann et al. (37) found that aurothioglucose and aurothiomalate were inhibitors of PKCα–Par6 interactions and slowed the growth of human A549 NSCLC xenograft tumors.

Clark et al. (38) found PKCδ to be antiapoptotic in NSCLC cells. Ding et al. (39) identified PKCδ as critically involved in drug-resistance in lung cancer cells by preventing cell from undergoing apoptosis. PKCα and PKCε work cooperatively in lung cancer cellular response to various stimuli (40). In cocultures of normal pulmonary fibroblasts and NSCLC cells, PKCα and PKCε, but not PKCδ, were critical to stromelysin-3 (MMP-11) expression, indicating the involvement of both classical and novel PKC in the regulation of stromelysin-3 expression (41).

An antisense agent designed to inhibit the production of PKCα has undergone clinical trials in NSCLCs (42). In preclinical studies, the antisense oligonucleotide, LY900003 (ISIS3521), inhibited PKCα mRNA and protein expression and had antitumor activity. In early clinical studies, LY900003 had single-agent activity and was promising in combination, particularly in patients with NSCLC, which led to a phase III clinical trial of LY900003 in combination with either cisplatin and gemcitabine or carboplatin and paclitaxel. However, in the phase III setting, LY900003 did not add significantly to the chemotheraphy regimens.

Brain Tumors

In rat hippocampal H19-7 cells and rat pheochromocytoma PC12 cells, the extracellular signal-regulated kinase activation by the neurogenic agents, fibroblast growth factor and nerve growth factor, is PKCδ-dependent, whereas extracellular signal-regulated kinase activation in response to epidermal growth factor is PKCδ-independent. Inhibition of PKCδ by antisense or rottlerin, blocked neurite outgrowth induced by fibroblast growth factor and nerve growth factor in PC12 cells and by activated Raf in H19-7 cells, indicating the involvement of PKCδ in the neurogenic effects of fibroblast growth factor, nerve growth factor, and Raf. Epidermal growth factor activates mitogen-activated protein kinase through a PKCζ-dependent pathway involving phosphatidylinositol-3-kinase and PDK1 in H19-7 cells (43). PKC regulates the activation of Raf-dependent signaling from growth factors by phosphorylating the Raf kinase inhibitory protein, thereby relieving the inhibition of the Raf/mitogen-activated protein kinase signaling cascade (44).

Phorbol 12-myristate 13-acetate–stimulated activation of PKCs in U-251 glioblastoma cells resulted in the activation of both Akt and the mammalian target of rapamycin signaling pathways as well as an increase in cell proliferation. Increased PKCη expression correlated with increased phorbol 12-myristate 13-acetate–induced proliferation in U-1242 MG cells (45). In the glioblastoma cell lines (U-1242 MG and U-87 MG), phorbol 12-myristate 13-acetate–induced epidermal growth factor receptor phosphorylation was blocked by bisindolylmaleimide, a PKC inhibitor, and rottlerin, a PKCα–specific inhibitor (46). Thus, phorbol 12-myristate 13-acetate activates the epidermal growth factor receptor and increases cell proliferation by activating the PKCζ/c-Src pathway in glioblastomas.

Multiple Myeloma, Leukemias, Lymphomas

PKC is directly involved in modulating apoptosis hematopoietic neoplasms. PKCδ, PKCα, PKCζ, and PKCε expression was detected in multiple myeloma cell lines (U266, RPMI-8226,
and K620; ref. 47). Using immunohistochemistry, PKCγ expression was confirmed in plasma cells from 11 patients. Alkan et al. (48) investigated PKC isoform expressions in chronic lymphocytic leukemia cells from seven patients and found the expression of PKCα, PKCγ, PKCδ, and PKCζ by Western blot in all of the samples.

**Disease Summary**

Several PKC isoforms including α, βII, ε, ι, and ι seem to be pro-oncogenic in the skin, colon, prostate, ovarian, breast, lung, and brain. PKCδ seems to have some possible tumor-inhibitory characteristics except in the brain, where it is more pro-oncogenic. Upstream and downstream pathways from PKCα and PKCδ are shown in Fig. 2. Many factors related to proliferation and invasion have been connected with PKCα; however, the pathways downstream from PKCδ form a less clear pattern. Several additional PKC isoforms have been identified in malignant tissues; however, no active role in the disease process has been elucidated thus far.

**PKC Inhibitors**

Small molecule approaches to inhibiting PKC have been directed toward the ATP-binding site or the diacylglycerol binding site. Varied levels of isoform selectivity can be shown for many naturally occurring and synthetic PKC inhibitors. Conformationally constrained analogues of diacylglycerol-lactones with approximately the same binding affinity for PKC as phorbol esters were explored (49, 50). Safingol, the L-threo enantiomer of dihydrosphingosine, acts as a competitive inhibitor at the diacylglycerol-binding domain of the enzyme. Balanol analogues with benzophenone subunits were inhibitors of PKC and PKA (51). The advent of staurosporine-like bisindolylmaleimide structures opened up the field of inhibitors such as CGP41251, Go-6850, Ro-31-8220, and UCN-01, at the ATP-binding site of the enzyme (52, 53). The National Cancer Institute 60-cell line identified the PKC inhibitor, UCN-01, 7-hydroxy-staurosporine. UCN-01 was shown to inhibit the growth of many tumor types in vitro and in vivo (54). UCN-01 has undergone phase I and II clinical trials alone and in combination regimens (55, 56).

**Midostaurin (PKC 412).** The N-benzoylated staurosporine analogue, midostaurin, was originally identified as a PKC inhibitor but has subsequently been found to inhibit both tyrosine kinase and serine/threonine kinases (57). Midostaurin is a potent radiation sensitizer both in cell culture and human tumor xenograft studies through a phosphoinositide-3-kinase/Akt pathway (58). In B16F10 murine melanoma, midostaurin was antimitotic activity because of the inhibition of the invasive and/or platelet-aggregating activities of the melanoma cells (59).

In culture, midostaurin was more cytotoxic toward B cell chronic lymphocytic leukemia than toward normal B cells (60). Exposure to midostaurin sensitized human U1810 NSCLC cells to DNA damage by etoposide (61). Midostaurin suppressed Akt kinase activation and induced apoptosis in human multiple myeloma cells (62).

Midostaurin is a potent inhibitor of mutant FLT3 and of the abnormal fusion protein, ZNF198-fibroblast growth factor receptor 1, as well as mutant KIT tyrosine kinase activities (63, 64). Gleevec-resistant disease remains sensitive to midostaurin (65). In culture, exposure of human leukemia cells to rapamycin, along with midostaurin, resulted in synergistic inhibition of cells expressing midostaurin-sensitive or -resistant leukemogenic FLT3 mutants. Similarly, the exposure of human acute myelogenous leukemia cells with mutant FLT3 to 17-allylamino-demethoxygeldanamycin or histone deacetylase inhibitor, LAQ824, and midostaurin resulted in increased cell killing compared with either agent alone, and relatively increased cell killing compared with acute myelogenous leukemia cells expressing normal FLT3 (66, 67).

Midostaurin has undergone several phase I clinical trials as a PKC inhibitor and as a tyrosin kinase inhibitor. Phase I single agent studies showed that midostaurin can be safely
administered by regular oral therapy. Phase I studies of midostaurin in combination with protracted continuous infusion of 5-fluorouracil in advanced solid tumors, in combination with paclitaxel and carboplatin, or gemcitabine and cisplatin in advanced NSCLC, established midostaurin doses that could be safely administered in these regimens (68, 69). Although phase II trials of midostaurin alone and along with imatinib in mutant FLT3 acute myeloid leukemia or gastrointestinal stromal tumors have shown activity, a mutation in the FLT3 tyrosine kinase domain which confers resistance to midostaurin has been identified (70).

**Enzastaurin (LY317615).** Enzastaurin is a potent inhibitor of PKCδ (71). Endogenous PKCδ mRNA levels in HCT116 cells were significantly reduced by enzastaurin, consistent with the effect on PKCδ promoter activity (72). Exposure to enzastaurin (600 nmol/L, 72 hours) profoundly inhibited the proliferation of vascular endothelial growth factor (VEGF; 20 ng/mL)–stimulated human umbilical vascular endothelial cells. When human SW2 small cell lung carcinoma cells were exposed to enzastaurin for 72 hours, a potency differential on the malignant cells versus human umbilical vascular endothelial cell was apparent. Cell culture studies indicate that exposure to enzastaurin can cause growth inhibition and apoptosis in human multiple myeloma cells, diffuse large cell lymphoma, and mantle cell lymphoma (73–76).

Enzastaurin at 10 mg/kg orally twice per day for 10 days post-surgical implant of VEGF-impregnated filters resulted in markedly decreased vascular growth to about one-half of the VEGF-stimulated controls, whereas 30 mg/kg decreased vascular growth to the level of surgical control (71). Enzastaurin at 30 mg/kg orally twice per day for 10 days post-surgical implantation of basic fibroblast growth factor resulted in decreased vascular growth to 26% of the basic fibroblast growth factor control. When nude mice bearing human tumor

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**Fig. 4.** A, survival of animals bearing intracranial human T98G glioblastoma multiforme after treatment with enzastaurin (3, 10, or 30 mg/kg, orally) twice per day on days 12 to 30 alone or with BCNU (15 mg/kg, i.p.) on days 7 to 11. B, survival of animals bearing intracranial human T98G glioblastoma multiforme after treatment with enzastaurin (10 or 30 mg/kg, orally) twice per day on days 4 to 18 alone or with BCNU (15 mg/kg, i.p.) on days 7 to 11. C, growth delay of s.c. T98G glioblastoma multiforme after treatment with enzastaurin (10 or 30 mg/kg, orally) twice per day on days 4 to 18 alone or with BCNU (15 mg/kg, i.p.) on days 7 to 11. D, growth delay of s.c. T98G glioblastoma multiforme after treatment with enzastaurin (3, 10, or 30 mg/kg, orally) twice per day on days 12 to 30 alone or with BCNU (15 mg/kg, i.p.) on days 7 to 11. Points, means of five animals; bars, ± SE (80).
enzastaurin was treated with enzastaurin orally, twice daily for 2 weeks, the number of intratumoral vessels was decreased to one-half to one-quarter of the controls in treated animals (Fig. 3A; refs. 71, 77–80). Although some tumors responded to enzastaurin as an antiangiogenic agent, in no case was angiogenesis completely blocked as in the corneal micropocket neangiogenesis model. The tumor growth delay did not correlate with intratumoral vessel decrease (Fig. 3B). In most tumor models, the tumor growth delay produced by enzastaurin as a single agent was not sufficient to predict single agent activity in the clinic. However, the combination regimens suggested high activity. VEGF plasma levels in mice bearing the human SW2 SCLC and Caki-1 renal cell carcinomas treated or untreated with enzastaurin were obtained every 3 days starting on day 7 post-implantation, throughout treatment, and after the termination of treatment. Plasma VEGF levels were similar between the treated and untreated groups up to day 20 (81, 82). Plasma VEGF levels in the control groups continued to increase throughout the study; however, even after termination of enzastaurin treatment, plasma VEGF levels in enzastaurin-treated mice were significantly decreased (83).

Simultaneous and sequential treatment regimens combining treatment with enzastaurin and 1,3-bis(2-chloroethyl)-1-nitrosoarene (BCNU) were studied in human T98G glioblastoma multiforme using tumor growth delay as the end point when the tumor was implanted s.c., and increase-in-life span as the end point when the tumor was implanted intracranially (80). For the simultaneous treatment regimen, enzastaurin was administered on days 4 to 18, and BCNU was administered on days 7 to 11 after tumor implantation. In this tumor model, treatment with enzastaurin produced tumor growth delays of 6.0 and 8.6 days at doses of 10 and 30 mg/kg, respectively (Fig. 4B). Administration of the combination of enzastaurin (30 mg/kg) with BCNU resulted in a tumor growth delay of 15.2 days.

Nude mice implanted with T98G (10^4 cells) intracranially had a mean survival time of 37 days. Administration of enzastaurin (30 mg/kg) alone doubled the survival time, producing a mean survival time of 72 days (Fig. 4A). Administration of BCNU to mice bearing the intracranial tumor implant resulted in a mean survival time of 41 days. The simultaneous combination regimen of BCNU with enzastaurin (10 or 30 mg/kg) produced mean survival times of 90 and 102 days, respectively (80).

Enzastaurin efficacy in the sequential treatment regimen after completion of BCNU was examined over a dosage range from 3 to 30 mg/kg. Enzastaurin alone on days 12 to 30 produced tumor growth delays of 18.5 to 32.6 days, respectively, in the s.c. T98G tumor (Fig. 4D). BCNU treatment followed by enzastaurin (30 mg/kg) resulted in a tumor growth delay of 46.8 days. Delaying treatment with enzastaurin (3-30 mg/kg) from days 4 to 12 after intracranial T98G tumor implantation produced mean survival times of 57 to 72 days compared with 37 days for controls (Fig. 4C). BCNU treatment followed by enzastaurin (3-30 mg/kg) resulted in mean survival times of 63 to 74 days compared with 41 days for BCNU alone (80).

Phase I clinical studies for enzastaurin were completed in 2003 (84, 85). Monocytoid collected from patients treated with enzastaurin showed decreased PKC activity after ex vivo stimulation, with phorbol ester providing a potential biomarker in a surrogate cell (86). Phase II studies have been conducted in patients with relapsed diffuse large B cell lymphoma and in patients with recurrent high-grade gliomas. Several patients with multiple relapsed diffuse large B cell lymphomas achieved prolonged periods of stable disease following enzastaurin treatment, although the objective tumor response rate was low (87). In patients with high-grade gliomas, enzastaurin was well tolerated and seemed to have promising antitumor activity in a significant percentage of highly pretreated patients (88, 89). In early combination studies, enzastaurin has proven safe and potentially efficacious along with capcitabine, pemetrexed, and gemcitabine and cisplatin (90–92). A phase III randomized, open label registration trial of enzastaurin in comparison with lomustine in glioblastoma has been initiated. A second phase III trial has been initiated in late-stage non–Hodgkin’s lymphoma where enzastaurin will be evaluated as a maintenance therapy in patients with diffuse large B cell lymphoma who have achieved remission following first line therapy to prevent relapse.

In summary, among the kinases being targeted with small-molecule inhibitors, the PKC family is perhaps the most ubiquitously expressed and centrally involved in cellular metabolism. While the clinical trials progress, science will continue to elucidate the spectrum of kinases effected by these agents and useful therapeutics will emerge.

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Protein Kinase C as a Therapeutic Target

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