Clinical and Radiographic Response in a Minority of Patients with Recurrent Malignant Gliomas Treated with High-Dose Tamoxifen

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PREVIOUS WORK HAS demonstrated the importance of the Protein Kinase C (PKC) signal transduction system in regulating the growth rate of malignant gliomas in vitro. Tamoxifen inhibits PKC in a minority of malignant gliomas within the micromolar concentration range in vitro, a property distinct from its estrogen receptor blockade effect. Tamoxifen was administered orally in very high dosages to 11 patients (9 males:2 females, age range 26-73, mean 45 years) with malignant gliomas (anaplastic astrocytoma or glioblastoma multiforme) who had failed treatment with external beam radiation therapy (and additional chemotherapy in 2). The dosage administered was estimated to be that necessary to achieve tissue concentrations within the low micromolar range, shown necessary to inhibit PKC in these tumors in vitro, and is approximately 5 times that used for standard antiestrogen therapy. Tumor reduction on radiographic images (MRI and PET [18FdG uptake]) with clinical improvement occurred in 3 patients; halting of tumor progression clinically and radiographically occurred in an additional patient. Of the remaining seven patients, three patients had marked and rapid progression of their disease despite treatment (dead after 3, 4, and 6 months respectively). Complications of treatment included a deep venous thrombosis requiring anticoagulation in one patient, nausea in one patient, and "hot-flashes" in a third patient. Tumor biopsy and measurement of tamoxifen and its active metabolite within the tumor of one patient (non-responder) showed levels within the middle of the in vitro therapeutic range. Follow-up of alive patients ranges from 4-18 months (mean 10 months). These encouraging preliminary results in a minority of these patients suggests some potential for this type of therapy. If PKC is the true target for any beneficial effect from tamoxifen therapy, this emphasizes the importance of clinical trials using other existing PKC inhibitors with increased potency and specificity for PKC. (Neurosurgery 32: 485–490, 1993)

Key words: Brain neoplasm, Chemotherapy, Glioma, Protein kinase C, Tamoxifen

Previous work has demonstrated that the proliferation rates of malignant gliomas are sensitive to modulators of Protein Kinase C (PKC) in vitro (1,3-7,17). Malignant gliomas express very high PKC activity when compared to non-transformed glial cells (2-3 orders of magnitude increase), both in human and rat systems (4-7). This high activity correlates strongly with the proliferation rates of these tumors in vitro (4,6). Moreover, identified glioma mitogens such as Fibroblast Growth Factor (FGF) and Epidermal Growth Factor (EGF) enhance PKC activity, which correlates with the increase in 3H-thymidine uptake produced by these growth factors. Altering enzyme activity by down-regulation or direct inhibition reduces the tumor growth rate significantly in vitro (1,3-7,17). Collectively, these observations have supported an important role of the PKC system in regulating glioma growth and have led to the speculation that PKC inhibitors may be utilized as adjuncts in the therapy of patients harboring malignant gliomas.

Tamoxifen, in a property distinct from estrogen receptor blockade, also inhibits PKC in the micromolar concentration range in these cells (1). An initial study administering low-dosage

tamoxifen (40 mg/day) to patients with recurrent malignant gliomas, while failing to demonstrate statistically increased survival, has documented isolated cases with apparent response to the treatment (19). As the growth and PKC inhibitory response to tamoxifen is dose-dependent (1,16), the following study was undertaken to assess the clinical safety and possible efficacy of administering very high dosages of tamoxifen to patients with recurrent malignant glial tumors. We report a clinical and radiographic (MRI and PET) response in a minority of patients administered high dosages of oral tamoxifen.

CLINICAL MATERIAL AND METHODS

Patient Selection

Criteria for inclusion in this study included A) patients ages between 18 and 75 years; B) histologically verified malignant glioma (anaplastic astrocytoma or glioblastoma multiforme); C) clinically and radiographically documented progression or recurrence following radiation (and chemotherapy in some cases); and D) no intercurrent illness, such as other malignancy, history of previous malignancy, blood dyscrasias, gynecological, ocular or gastrointestinal disease. From July 1991 to July 1992, 14 patients were chosen for inclusion in this study, of which 11 complied with treatment and were evaluable (Table 1; 9 males:2 females, age range 26-72, mean 45 years). Three patients which were not evaluable included 2 cases of non-compliance and one case of rapid deterioration and death within one month in a very ill patient with recurrent glioblastoma multiforme.

Method of Treatment

All patients were screened for compliance and acceptance criteria. All pathology was reviewed by one of the authors (DH). Baseline radiographic images (MRI) and blood work (Complete Blood Counts [CBC] and Serum Chemistry) were performed prior to initiation of therapy. Tamoxifen (ICI Pharmaceutical, Wilmington, Delaware) was first administered at standard antiestrogen doses (20 mg orally BID) to observe for any side effects. If tolerated, the dose was increased weekly to achieve target dose over a 1 month period (80 mg BID in females, 100 mg BID in males). CBC and Serum Chemistry panel were performed every 2 weeks while escalating the dose and every 2 months thereafter while on the drug.

Observation and Evaluation

Patients included for evaluation included those who tolerated the drug at maximal dosages for a period of 2 months or longer, survived for at least one month following attainment of maximal therapy (to enable significant steady-state tissue levels of tamoxifen to be obtained), and were clinically and radiographically evaluated during the treatment period.

All patients underwent initial and serial MRI imaging with and without gadolinium enhancement. Initial tumor volume was estimated by measuring the cross-sectional diameters at the level of the largest contrast-enhancing tumor extent. These measures were multiplied, and the product was multiplied by the extent of maximal enhancement on coronal studies. Estimation of the tumor volume excluded areas of cystic change or edema. Subsequent comparison studies chose comparable MRI slices with tumor measurement by the same technique.

Serial PET scans were performed on 7 patients during treatment. Each subject received 10 mCi of 18F-fluorodeoxyglucose, and images of relative glucose metabolism were obtained 30-40 minutes following tracer injection. Image acquisition was performed over a 30 minute period using a Siemens 953-A tomograph. Plane thickness was 3.4 mm and reconstructed in-plane image resolution was 7 mm full width half maximum. All images were normalized to an equivalent index for normal cortex, to enable comparison of tumor site. For comparison studies, images were chosen to incorporate the region of maximal glucose metabolism, and were visually inspected for regional changes.

Treatment response (R) was defined as a greater than 50% decrease in volume of the enhancing lesion volume on MRI and a decrease in metabolic activity (18FdG uptake) on PET scans with clinical neurological improvement (including Karnofsky scores). Stabilization or no change (NC) was defined as <50% reduction in tumor volume radiographically and no clinical progression. Progressive disease (PD) included all other patients (worsening clinically or radiographically). Corticosteroid dosages in all patients were either maintained or decreased on comparison radiographic studies.

In one patient (A.J.), tumor tissue levels of tamoxifen and its active metabolite N-desmethyltamoxifen were measured in a snap frozen surgical tissue specimen by Normal-Phase High Pressure Liquid Chromatography (HPLC).

RESULTS

Side Effects

Three patients exhibited side effects during the course of their treatment. One patient developed nausea following dose escalation of tamoxifen. The persistence of the nausea prompted temporary withdrawal of therapy, with no resolution of the symptom. The drug was restarted and the symptom slowly resolved. Another minor complication was "hot-flashes" experienced by a young female just after initiation of therapy. One major complication encountered was a deep venous thrombosis in an elderly male on maximal therapy for a period of 12 weeks, which was managed by anticoagulation following discontinuation of therapy.

Response Rate

Table 1 summarizes the response and follow-up of the 11 evaluable patients on high dose tamoxifen therapy. Response (R) was noted in 3 patients, with improvement clinically and radiographically (Figures 2 and 3). The 2 responding patients shown in the illustrative cases both harbored glioblastoma multiforme, with one patient failing 2 previous clinical protocols of a) CCNU, Vincristine and Procarbazine, and b) Carboplatin chemotherapy. These 3 responders have all survived longer than 12 months, with clinical improvement. Another patient has demonstrated no change (NC) in clinical status and radiographic images over the follow-up period (6 months). Three patients had marked and rapid progression of disease while on therapy which resulted in death at 3, 4 and 6 months following initiation of therapy (Figure 1). The remainder of the non-responding cohort has demonstrated either clinical or radiographic deterioration over the follow-up period.

Measurement of tamoxifen and its active metabolite

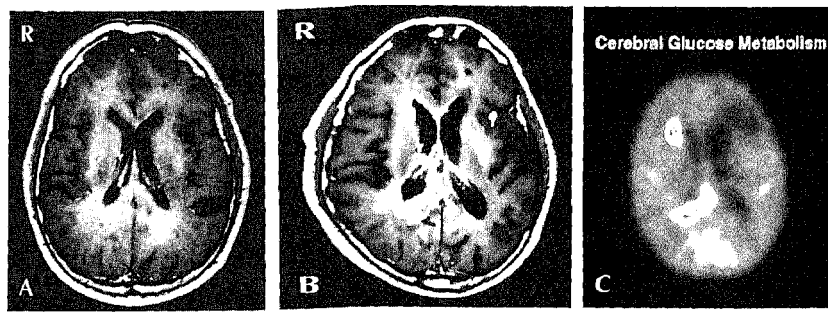

FIGURE 1. This 68-year-old male presented with a vaguely enhancing right parietal mass (A) which revealed anaplastic astrocytoma by stereotactic biopsy. Recurrence of tumor occurred shortly following radiation therapy, and he was placed on high dose tamoxifen therapy. The lesion quickly progressed clinically over the ensuing weeks, as documented by MRI (B) and PET scan (FdG uptake, C). Following the development of a deep venous thrombosis (DVT) the patient was discontinued from therapy and he succumbed to the tumor 16 weeks following the start of therapy. Autopsy revealed glioblastoma multiforme.

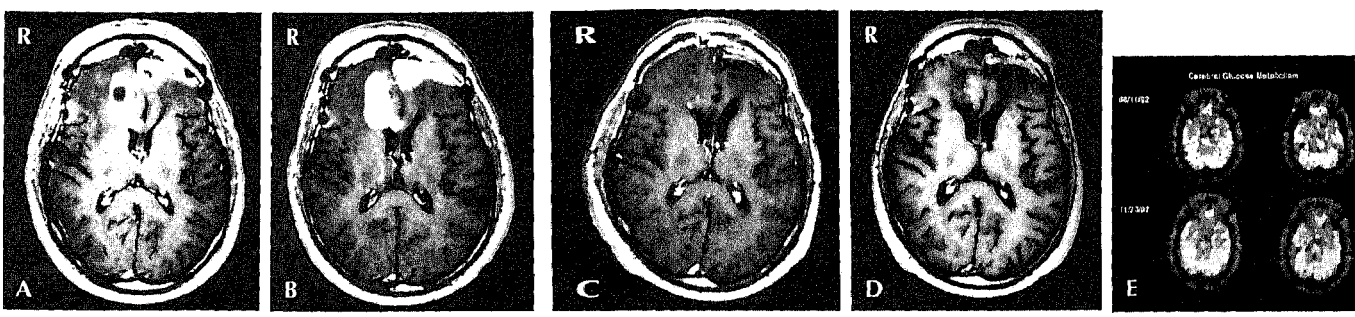

FIGURE 2. A 49-year-old male, with recurrent bifrontal butterfly glioblastoma 7 months following partial surgical resection and external beam radiotherapy. The patient had been initially started on tamoxifen 40 mg/day, with slight radiographic progression of tumor despite therapy (comparison of the November (A) and December (B) MRI study performed 6 weeks later demonstrates increased enhancement and further extension through the corpus callosum). Dosage was increased to 100 mg BID, which was followed by a marked decrease in size of enhancing tumor on MRI 2 months following (C). Follow-up studies 9 months later indicate no change in the scans (D). The patient was never placed on corticosteroid therapy during the course of treatment. The small residual enhancing lesion on the MRI continues to show a decrease in metabolic activity on later serial PET images (E). The patient is neurologically intact.

N-desmethyltamoxifen in the tumor of one non-responding patient (A.J.) who underwent a temporal lobectomy showed levels of 2.6 and 11 micromolar respectively.

Median survival for the entire cohort was 6 months, with a mean length of follow-up of 8.4 months (36 weeks). Mean length of follow-up of alive patients is 10 months (43 weeks).

Radiographic Observations

Radiographic response is shown in Table 1. The patients who had regression of enhancement on MRI images also demonstrated a decrease in metabolic activity on PET scans (Figures 2 and 3). The patient who has remained clinically unchanged has had a halting of progression on MRI scan. All other patients demonstrated progressive disease either on PET or MRI studies.

DISCUSSION

Intracellular transmission of growth factor signals is presumed to be mediated by a network of sequentially activated protein kinases. Protein Kinase C (PKC) has been implicated in the transduction of cellular responses to various agonists including hormones, neurotransmitters, and some growth factors in selected cell types (11-13). In this regard, recent reports have identified some mitogenic growth factor signals which may be transduced through PKC in malignant gliomas and non-transformed glial cells (6,20). The observations of high PKC activity in frozen surgical malignant glioma tissue (6), together with the demonstrated strong coupling of PKC activity and proliferation rate in vitro (4,6), suggest that this enzyme activity may function as a chemotherapeutic target in vivo.

Tamoxifen has previously been shown to inhibit PKC in other cell types (14,15) and to decrease the proliferation rates of established glioma cell lines in vitro (1,16). It also inhibits approximately 25-30% of low-passage glioma specimens in vitro (1). The results from the present study indicate that high-dose oral tamoxifen appears to be well tolerated in most patients, and may inhibit tumor growth in a minority of patients with recurrent malignant gliomas. This response in a patient to high-dose tamoxifen has also recently been noted by another group (2, Yong, V.W. and Baltuch, G., McGill University, personal communication). The concentration of tamoxifen necessary for PKC inhibition in these cells lies within the micromolar range in vitro

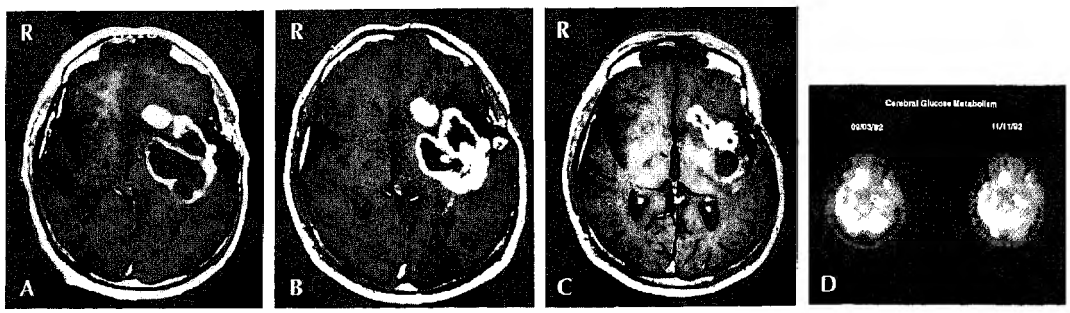

FIGURE 3. This 38-year-old male had deteriorated clinically and radiographically through 2 experimental chemotherapy protoco with nitrosureas and carboplatin for treatment of glioblastoma over a period of 9 months since the tumor was first diagnosed. Clinically he had developed a rapidly progressing expressive aphasia with right hemiparesis. MRI scan revealed the cystic left frontc temporal mass prior to tamoxifen therapy (A). Following surgical drainage of his temporal cysts and resection of glioblastoma from the anterior temporal tip, the patient was started on high dose tamoxifen therapy. Subsequent postoperative MRI studies pe formed at 6 weeks (March; B) and 11 months (December; C) following initiation of therapy indicate regression of the enhancing tumor in the region of the basal ganglia and fronto-temporal region distant from the surgery; the patient's clinical status has improved markedly paralleling the radiographic changes. Serial PET studies also indicate a decrease in glucose metabolism withii these regions (D). He is presently 12 months duration on tamoxifen with continued clinical improvement, with total resolution of hemiparesis and marked improvement in expressive aphasia. He is currently on no corticosteroid therapy.

(1,16); measurement of the drug and its active metabolite in the tumor of one of the patients in our own study indicate that sizable dosages must be administered to achieve this, far greater than those used clinically for blockage of the estrogen receptor. This dose-dependency was illustrated by one patient (H.G.; Figure 2), who was progressing on low-dose therapy yet responded to high-dose tamoxifen. Lack of toxicity with this type of therapy makes it particularly appealing for use in patients with extensive malignant disease, despite the fact that extended therapy is necessary due to its "tumoristatic" nature (ie., it slows or halts tumor cell proliferation rather than killing the cell). Moreover, recent laboratory work has demonstrated it may also potentiate the effect of radiation therapy in malignant glioma cell possibly also mediated by a PKC-dependent mechanism (21).

Of interest is the clinical and radiographic response of on patient (J.B.; Figure 3) in the present study who had previousl failed 2 chemotherapeutic drug protocols, indicating that th type of treatment may prove suitable as salvage therapy follow ing standard chemotherapy in some patients.

Prediction of the tumors that may respond to this type of the apy is unknown at present. However, a potentially importar observation is that the percentage of patients that appear t respond to high dose tamoxifen therapy is similar to the nun ber of low-passage glioma lines that are sensitive in vitro (1), sug

TABLE 1. Clinical Data

Case	Sex, Age in yrs.	Pathology[a]	Tumor Location	Pre-Treatment Karnofsky	Previous Therapy[b,c,d]	Tamoxifen Dose (mg/day)	Radiographic Response[e]	Improvement of Symptoms[f]	Post-Treatment Karnofsky	Survival Period (mos
H.G.	M, 49	Glioblastoma	Bifrontal	90	surg, rad	200	R	+	100	18
A.J.	F, 36	Glioblastoma	L-Temporal	60	surg, rad	160	PD	–		6†
D.T.	M, 37	A. Astrocytoma	R-Fronto-Parietal	60	rad	200	R	+	70	15
J.B.	M, 38	Glioblastoma	L-Fronto-Temporal	70	surg, rad, chemo	200	R	+	80	12
J.Z.	M, 68	A. Astrocytoma	R-Parietal	70	rad	200	PD	–		4†
A.S.	M, 42	A. Astrocytoma	R-Frontal	60	rad	200	PD	–		10†
E.P.	F, 73	Glioblastoma	R-Frontal	50	surg, rad	160	NC	±	50	6
M.V.	M, 35	Glioblastoma	Cerebellar	80	surg, rad	200	PD	–	70	6
D.M.	M, 26	A. Astrocytoma	R-Parietal	90	rad	200	PD	±	90	8
L.D.	M, 56	Glioblastoma	L-Parietal	40	surg, rad	200	PD	–		3†
R.S.	M, 36	A. Astrocytoma	L-Parietal	60	surg, rad, BCNU	200	PD	–	50	4

[a]A.= Anaplastic
[b]surg = craniotomy with tumor reduction; all others underwent stereotactic biopsy only
[c]rad = external beam radiation therapy
[d]chemo = chemotherapy (1: CCNU, Vincristine, Procarbazine, 2: Carboplatin)
[e]R = response, NC = no change, PD = progressive disease
[f]+ = improvement, ± = equivocal, – = negative
[g]† = death

gesting that in vitro-sensitivity testing may be feasible. The presence of the amplified EGF-receptor (EGFR) and its gene noted in a significant proportion of glioblastoma specimens (8,9), and the observation of the increased response of patients harboring glioblastomas as compared to anaplastic astrocytomas in the present study suggest the interesting hypothesis that this amplified receptor may increase PKC activity and thus tamoxifen sensitivity in these patients (6).

The definition of the true efficacy of high-dose tamoxifen in patients harboring malignant gliomas is not possible from this limited pilot study and will require larger Phase II trials. However, if PKC is the target for any beneficial effect in these patients, then future clinical work should focus upon administration of more specific and/or potent PKC inhibitors which already exist (4,6,10). For example, whereas tamoxifen inhibits only a minority of low-passage glioma lines in vitro, the potent (though only relatively specific) PKC inhibitor Staurosporine inhibits all glioma tumors tested within the low nanomolar range (1,4,6), suggesting agents such as this may prove to be more favorable for the treatment of these lesions. Intratumoral administration of this agent or a derivative has been shown to inhibit tumor growth in an in vivo glioma model (unpublished data) and systemic therapy to inhibit the growth and invasion of other in vivo tumors (10,18).

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COMMENTS

There has been considerable interest in finding new biochemical pathways which are involved in controlling the growth of malignant gliomas. The Protein Kinase C pathway has been of particular interest. This is in part because of the fact that there are a variety of potential therapeutic agents which can modulate this pathway. Of these agents, tamoxifen is perhaps the most clinically relevant at the present time.

In this paper, the results of a clinical trial of tamoxifen in a small series of glioma patients appears to be encouraging and form the basis for making further studies of the potential value of this concept.

It is of particular interest that an agent such as tamoxifen would play a therapeutic role inasmuch as it does not have many of the toxic side effects of standard chemotherapy agents and is therefore a potentially valuable approach as an alternate to alkylating agents and even as an adjunct to cytotoxic chemotherapy.

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The authors describe the clinical effects of high-dose tamoxifen on 11 patients with recurrent glioblastomas. A positive response was obtained in a minority of the patients treated with high doses (3 of 11 cases). This kind of therapy is new in the field of neurooncology and this preliminary report is thus very important, as was the previous one on the same topic with lower doses (reference 19). Although this clinical trial is based on outstanding laboratory work on protein kinase C (PKC) activity in glioblastoma cells done by the first author, the mechanisms of action of tamoxifen deserve some comments.

1. Tamoxifen is known to have several biological activities including estrogen inhibiting action (antagonism of estrogen-receptor binding) (4); protein kinase C inhibition (by membrane-substrate protein interaction) (5); and inhibition of calmodulin-independent Ca^{2+} transport system (6). As mentioned by the authors, several studies suggest that tamoxifen inhibits the PKC activity of glioblastoma cells, however there is no direct proof that this was actually the mechanism of action in the patients.

2. Protein kinase C is a major pathway of signal transduction for many peptide growth factors and hormones, and is therefore necessary for the survival and growth of various normal cells as well as the neoplastic cells. Administration of a potent inhibitor of PKC might cause severe toxicity on hematopoiesis and even on the brain function (7), and should be done with caution.

3. Receptors for epidermal growth factor (2) and fibroblast growth factor (3,8) have tyrosine kinase domains and their signal transductions do not enhance PKC activity directly. EGFR is induced by estrogen receptor or transmodulated by PKC activity (1).

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This article by Couldwell et al. is interesting and provocative. However, the study is very small, with only 11 patients dispersed between two malignant histologies, anaplastic gliomas and glioblastomas multiforme. In addition, the median follow-up for the study is only 8.4 months. Under the circumstances, one cannot be certain of the true benefit of tamoxifen.

This study, while showing some activity, has insufficient data upon which to judge true efficacy of this therapy. There are only hints of activity at this time, which are provocative and may in fact be harbingers of an important study.

I believe that the authors have to conduct a proper study and increase the number of patients to an appropriate number for phase II studies of this kind. Normally, most statisticians, once activity has been demonstrated, will require somewhere between 17 and 20 patients for each histology to get good confidence intervals on the relative activity of the study. There needs to be a more careful analysis of anaplastic astrocytoma vs. glioblastoma multiforme with attention to response rates and duration of response.

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With recent advances in the understanding of basic tumor biology, it is now apparent that tumors originate and progress because of abnormal regulation of normal cellular biochemical and physiological processes. A common feature of abnormal cell biology in cancer is that of an alteration in one or more of the pathways by which external signals, such as growth factors, are conveyed to the intracellular constituents, the signal transduction pathways. This recognition provides multiple new targets for anticancer treatment and, thus, permits the potential of developing therapies that do not simply depend on direct interaction of the therapeutic agent with production of DNA by the tumor, as is the case with most forms of standard chemotherapy.

Protein kinase C, an enzyme activated by receptor-mediated hydrolysis of inositol phospholipids, relays information in the form of a variety of extracellular signals across the cell membrane to regulate many Ca^{2+} – dependent processes. Protein kinase C is activated, as assayed by the phosphorylation of its endogenous substrates, by tumor promoting agents, such as the phorbol esters, and has enhanced activity in malignant gliomas. Couldwell et al. demonstrate clear evidence of antitumor activity of tamoxifen, an inhibitor of protein kinase C, in patients with recurrent malignant gliomas, with minimal associated toxicity. Not only is this observation important because of the potential benefit that such treatment may have alone, but also for the potential of using this, or other, more potent, inhibitors of protein kinase C, combined with conventional therapies, or combined with other new agents that selectively target additional points of vulnerability that have been exposed by basic research in tumor cell biology, for more effective tumor control and less toxicity than the limited choices now available.

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