Enzastaurin, a Protein Kinase Cβ–Selective Inhibitor, and Its Potential Application as an Anticancer Agent in Lung Cancer

Roy S. Herbst,1 Yun Oh,1 Asavari Wagle,2 and Michael Lahn2

Abstract Enzastaurin, an oral serine/threonine kinase inhibitor, suppresses signaling through protein kinase C (PKC)–β and the phosphatidylinositol 3-kinase/AKT pathway to induce tumor cell apoptosis, reduce proliferation, and suppress tumor-induced angiogenesis. In contrast to previous PKC inhibitors, enzastaurin is very well tolerated with a favorable safety profile, allowing it to be dosed for extended durations. In the present review, we summarize the rationale for targeting PKC in cancer, the preclinical experience of enzastaurin, and the clinical findings of the current phase I and II studies. Based on the combined information, we present the rationale for its future assessment in the treatment of lung cancer.

The protein kinase C (PKC) family of isoenzymes (1), which belongs to the widely expressed group of serine/threonine kinases, is involved in key cellular processes including cell proliferation, apoptosis, and differentiation (2). The different isoforms of PKC exert positive and negative effects on cell proliferation and survival, and altered levels of individual isoforms are implicated in transformed cells and other disease states (3, 4). In cancer, PKCs may affect tumor formation and progression (5) by virtue of their function as receptors for carcinogens, such as phorbol esters, and as second messengers for growth factors, such as vascular endothelial growth factor. PKC activation has been shown to increase invasiveness of cancer cells by its effects on cell adhesion, barrier migration, and matrix degradation (6–8). Recently, PKC has also been implicated in chemoresistance (9). PKC overexpression and increased activity has been linked to many cancers including colon, renal cell, hepatocellular, non–small cell lung, and prostate (10–14). The actual mechanism by which PKC contributes to tumorigenesis is unclear. Recent evidence suggests a link between PKC and phosphatidylinositol 3-kinase/AKT, the main pathway responsible for apoptosis regulation (15, 16). As shown in Fig. 1, PKC and AKT can both phosphorylate glycogen synthase kinase (GSK)-3 (17, 18), and this overlap of the PKC and phosphatidylinositol 3-kinase/AKT pathways may be a mechanism by which PKCs regulate tumor cell apoptosis. Based on their tissue expression and substrate/activator specificity, the individual PKC isoforms may exert differential control on cell proliferation/apoptosis, angiogenesis, and tumor invasiveness. The PKCβ isoform is known to be an important mediator of vascular endothelial growth factor (19, 20), the most potent angiogenic factor in the highly vascular brain, kidney, bladder, and ovarian tumors. Increased invasion and proliferation has also been associated with PKCβ (8, 21). In diffused large B-cell lymphoma, PKCβ is one of the most prominently overexpressed genes and is linked to poor prognosis (22, 23). Thus, based on the multifunctional role PKC plays in cancer, inhibition of individual PKC isoforms may provide new opportunities in improving the clinical outcome of cancer treatment.

Characterization of Enzastaurin in Preclinical Models

Enzastaurin, initially developed as an ATP-competitive selective inhibitor of PKCβ (24), also targets the phosphatidylinositol 3-kinase/AKT pathway and inhibits GSK3β (Ser9) and ribosomal protein S6 (Ser240/244) phosphorylation (25). In vitro preclinical assays show ~95% plasma protein binding and an IC50 of 70 nmol/L for PKCβ. Enzastaurin exhibited proapoptotic and antiproliferative activities in various cancer cell lines (26). Recently, Nakajima et al. (27) showed that enzastaurin inhibited the growth of a panel of 11 small-cell lung cancer lines (IC50s 3-10 µmol/L) and 4 non–small cell lung cancer (NSCLC) cell lines (IC50s 3-10 µmol/L) and reduced phosphorylation of GSK3β. Vascular endothelial growth factor expression and microvessel density in human tumor xenografts were decreased in the presence of enzastaurin (28). In rat corneal micropocket assay, 30 mg/kg enzastaurin (free drug = 100 nmol/L) suppressed the growth of new blood vessels (29). In animal models, enzastaurin showed antitumor and antiangiogenic activities in various malignancies, including colon, renal cell, and hepatocellular carcinomas and NSCLC (30, 31). Enzastaurin suppressed tumor-induced angiogenesis in A549 NSCLC xenografts (32). In xenograft models of glioblastoma (U87MG) and colorectal cancer (HCT116), enzastaurin showed a tumor growth delay, which is associated with a reduction in GSK3β phosphorylation (25). Hence, GSK3β may have utility as a potential marker of downstream...
activity of enzastaurin in tumor or surrogate tissue (25). The antitumor effect of enzastaurin was also confirmed by subsequent studies using patient-derived tumor explants (33). These explants are maintained as xenografts and retain most of their original histologic characteristics. Hence, these xenografts seem to better predict future antitumor activity of new anticancer drugs in clinical investigation. Using a standardized clonogenic assay (34), enzastaurin was applied at a continuous exposure of doses, ranging between 0.001 and 100.0 μmol/L, in a panel of 51 different xenografts. Enzastaurin showed both antitumor activity and selectivity. Selectivity was particularly observed against tumor xenografts of leukemia, lymphoma/myeloma, small-cell lung cancer, and melanoma. In addition to these panels of either tumor cell cultures or clonogenic assays, enzastaurin has been evaluated in a number of hematologic cell culture systems, such as lymphoma, multiple myeloma, cutaneous T-cell lymphoma, and mantle cell lymphoma (35–39).

Enzastaurin has also been evaluated in combination with other anticancer agents in cell lines and freshly isolated human tumor biopsy material. The combination of pemetrexed with enzastaurin was found to be synergistic in thyroid cancer cells (40). An additive and even synergistic effect was observed in NSCLC and small-cell lung cancer cell lines (27, 41). Finally, enzastaurin was found to exert antitumor activity in taxane-resistant ovarian cancer cells, suggesting unique activity in drug-resistant tumor cell lines (42). In summary, data from previous and ongoing preclinical studies indicate that the activity of enzastaurin is not limited to the tumor microenvironment alone (e.g., changes in the tumor vasculature). Enzastaurin acts on both the endothelial cell and the tumor cell to suppress tumor growth through multiple mechanisms: inhibition of cell proliferation, induction of cell death, and inhibition of tumor-induced angiogenesis.

**Early Clinical Investigation of Enzastaurin**

Based on the preclinical information about its mechanism of action and its widely shown antitumor activity, enzastaurin was investigated in a phase I dose escalation study (JCAD) in patients with advanced, solid tumors (43). Adenocarcinoma of the lung was the most common diagnosis. Determination of the recommended phase II dose was based on data for toxicity and pharmacokinetics, with the intent of exceeding effective plasma exposures. The targeted mean steady-state total concentration for clinical efficacy is ~1,400 nmol/L (based on preclinical data). This steady state is achieved within 2 weeks of daily oral dosing. During this study, no maximum tolerated dose was identified. A dose of 525 mg/d produced mean steady-state plasma concentrations of ~2 μmol/L and did not result in unacceptable toxicity. Plasma exposures did not increase with increasing dose and, therefore, 525 mg/d was identified as the recommended phase II dose (525-mg capsules equivalent to the new 500-mg tablet). Mouse xenograft-suppressing activity has since been shown retrospectively at plasma exposures similar to those observed in this study (25). The oral dosing regimen was well tolerated at all doses (maximum dose, 700 mg) with no clinical grade 3 or 4 toxicities. This remarkable toxicity profile was associated with stable disease as best response in 21 (45%) patients, including 4 lung cancer patients, and the time on enzastaurin ranged from 2 to 16 cycles (1 cycle = 28 days). During this and subsequent studies, an attempt was made to characterize the inhibition of PKCβ in surrogate tissue. The approach was based on using flow cytometric assessment of PKC substrate phosphorylation to determine the inhibition of these substrates at steady-state levels (44). The inhibition of PKC substrate phosphorylation in peripheral blood mononuclear cells was achieved at the target steady-state level of 1,400 nmol/L.
nmol/L. Because this assay requires immediate flow cytometric evaluation, it was difficult to carry this assessment into subsequent trials.

Subsequent to this phase I study, combination studies with other chemotherapeutics were conducted (Table 1). One study focused on several aspects of the combination with pemetrexed and enzastaurin (45, 46). In this study, no drug-drug interaction between enzastaurin and pemetrexed was observed. Long-term treatment with the combination revealed that enzastaurin did not give additional toxicity to the known toxicity profile of pemetrexed. Another combination study assessed the safety and pharmacokinetics of enzastaurin when combined with cisplatin and gemcitabine (47). In this study, 33 patients were treated in a dose escalation design in which enzastaurin was escalated and gemcitabine and cisplatin were administered at standard doses. There was no significant change in the pharmacokinetics of any of the three agents, suggesting a predictable exposure of enzastaurin, cisplatin, and gemcitabine. The toxicities seen in the study were those expected with the gemcitabine/cisplatin combination. Partial responses were seen in three patients with pancreatic, head and neck, and ovarian cancer. In addition to these phase I studies examining chemotherapies approved in the treatment of advanced and metastatic lung cancer, another phase I study was conducted to determine the combination of enzastaurin with capcitabine (48). As in other studies, the pharmacokinetics and safety remained unchanged with the addition of enzastaurin, and some patients achieved stable disease for >6 months.

Based on these encouraging phase I data, early phase II studies were conducted in glioblastoma multiforme (GBM) and diffuse large B-cell lymphoma (DLBCL; refs. 49, 50). In both of these studies, enzastaurin was well tolerated and complete tumor responses were reported, suggesting that enzastaurin has single-agent activity in patients with advanced, relapsed cancer. Thrombocytopenia (1 patient, grade 3) was the only bone marrow toxicity observed in patients with DLBCL. In addition, enzastaurin exhibited single-agent activity, with 8 of 55 patients free from progression for ≥4 cycles of therapy and 4 patients free from progression for ≥12 months. In the GBM study, objective radiographic responses were noted in 23% of heavily pretreated patients, 5% of GBM patients, and 13% of AG patients stable for ≥3 months. Grade 3/4 thrombocytopenia was the only consistent adverse event and was observed in only 3% of the enrolled patients, whereas only 1 patient reported hepatic transaminase elevation grade ≥2. Potential efficacy in other tumor histologic types is currently being explored in additional single-agent phase II studies. For example, in NSCLC, a recent interim analysis of a phase II study in patients receiving enzastaurin as second- or third-line treatment estimated a 10.4% 6-month progression-free survival rate (51) and the most common toxicity was grade ≤2 fatigue. The final analysis of this trial is expected to provide additional information, as some of the patients had been censored at the time of the interim analysis. Thus, the data from the interim analysis should be interpreted with caution.

In a recent safety assessment of the combined phase I and II study populations, comprising a total of 135 patients, enzastaurin was shown to maintain a favorable safety profile (52). In the patient populations analyzed, no drug-related deaths were reported. No severe hepatic or cardiac toxicity was reported. The most common adverse event was grade 1 chromaturia due to the orange color of the pill. There were no reports of drug-related severe bone marrow suppression (grade 3 or 4 neutopenia, thrombocytopenia, or anemia). Other grade 3 adverse events, including edema, migraine, and peripheral motor neuropathy, were observed only in one patient each. Patients on therapy for <30 days reported the highest number of adverse events, consistent with the severity of their disease. Patients treated for >180 days did not experience any drug-related grade 3 or 4 toxicity. This review of the safety data confirmed the earlier observation that enzastaurin is well tolerated by cancer patients.

### Table 1. Phase I combination studies of enzastaurin

<table>
<thead>
<tr>
<th>Combination chemotherapy with enzastaurin</th>
<th>Daily dose of enzastaurin</th>
<th>Response</th>
<th>N</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capecitabine</td>
<td>350-525 mg</td>
<td>10 patients SD ≥2 mo; 5 patients SD ≥6 mo</td>
<td>27</td>
<td>Advanced cancer</td>
</tr>
<tr>
<td>Gemcitabine/cisplatin</td>
<td>350 mg qd-500 mg bd</td>
<td>3 PR; 13 patients SD ≥2 mo; 11 patients SD ≥6 mo</td>
<td>33</td>
<td>Advanced cancer</td>
</tr>
<tr>
<td>Pemetrexed (ongoing)</td>
<td>1,200 mg loading dose on day 1, 500 mg qd</td>
<td>2 PR; 36 patients SD ≥2 mo; 5 patients SD ≥6 mo</td>
<td>42</td>
<td>Advanced cancer</td>
</tr>
</tbody>
</table>

Abbreviations: PR, partial response; SD, stable disease.

### Potential Use of Enzastaurin in Lung Cancer

Based on its expression in lung cancer (53) and its functional role in lung cancer development (54, 55), PKCβ may be a therapeutic target in lung cancer. Whether targeting specific isoenzymes of PKC is a reasonable approach to treat lung cancer is unknown. For instance, aprinocarsen (or LY 900003; ISIS3521), a specific inhibitor of PKCα, failed to add significant survival benefit when added to chemotherapy regimen of carboplatin and paclitaxel (56) or gemcitabine and cisplatin in two separate phase III studies in patients with advanced NSCLC. However, the clinical development of aprinocarsen lacked some important translational information which may have better characterized the activity of this specific PKC inhibitor: first, patients in both studies were not selected based on their increased PKCa associated signaling; second, it was never established whether aprinocarsen really accumulated in the tumor tissue; and last but not least, the...
pharmacokinetic profile of aprinocarsen was not fully described in either of the large phase III studies. Currently, less specific PKCs are being developed, such as PKC415 (57). At this time, it remains unresolved whether less specific PKC inhibitors are more effective anticancer drugs than the specific PKC inhibitors, including enzastaurin. Although enzastaurin was originally developed as a rather specific PKC inhibitor, subsequent studies have shown that it has additional activities on other kinase targets. For example, at clinical plasma concentrations, enzastaurin can also inhibit PKCα. Because PKCβ and PKCα are both overexpressed in lung cancer (53, 58), it is possible that this broader activity of enzastaurin will be different than that observed for aprinocarsen. Furthermore, enzastaurin can suppress the phosphatidylinositol 3-kinase/AKT signal transduction, which is elevated in lung cancer, and inhibition of this pathway has recently been shown to enhance radiosensitization in NSCLC cell lines (59). Increased AKT phosphorylation due to loss of phosphatase and tensin homologue expression has been associated with poor prognosis in lung cancer (60). Based on these data and given the recent activities of enzastaurin in preclinical lung cancer models, initiation of clinical studies in NSCLC and small-cell lung cancer is justified. The first signal of activity of enzastaurin in NSCLC was observed during the initial phase I study (43), where four patients with advanced lung cancer were treated with enzastaurin for 3 to 12 cycles. The longest-treated NSCLC patient received enzastaurin for 12 cycles. The best response in all four patients was stable disease. Patients with head and neck cancer also seemed to benefit from enzastaurin therapy in this trial. Based on these observations, a single-agent, single-arm, three-institution study in second- and third-line NSCLC patients was initiated. The study has recently been completed, and data from the final analyses are expected to be presented soon. The interim analyses showed that 10 patients had no disease progression for >6 months and 3 of these patients continued treatment for >9 months. The most common toxicity was fatigue (n = 21), noted within 1 week of starting treatment, in patients who progressed, but this was not reported in patients with disease stabilization. These data are consistent with enzastaurin activity as a tumor growth inhibitory agent that does not show direct tumor cytotoxicity (37). Disease stabilization seems to be the likely activity of this drug in advanced and heavily pretreated NSCLC patients.

Conclusion

In summary, enzastaurin seems not to share the toxicity profile of most other anticancer drugs. It also seems to differentiate itself from other anticancer agents by its inhibitory activity on a number of proliferation-associated kinases such as phosphatidylinositol 3-kinase/AKT, GSK3, and S6 kinase, producing long-lasting tumor growth arrests in preclinical and clinical studies. The ease of administration, the favorable toxicity profile, and the evidence of activity as observed by long-term responses in both solid and hematologic cancers suggest that enzastaurin should be evaluated further as an anticancer agent. Recently published studies indicate that targeted agents work synergistically with cytotoxic agents (61, 62). Hence, given the distinct toxicity profile and molecular targets, a combination therapy with enzastaurin may be a novel approach in the treatment of cancer. Enzastaurin is currently being evaluated in phase III studies for glioblastoma and non-Hodgkin’s lymphoma. Additional studies in NSCLC combining enzastaurin with other anticancer agents for NSCLC are being planned.

Open Discussion

Dr. Thomas Lynch: With a drug like this, do we say it doesn’t work, cut your losses, move on? Or should further preclinical modeling be done to establish if enzastaurin is worth further study in lung cancer?

Dr. John Heymach: What would persuade me that this should be combined with chemotherapy is either really dramatic synergy or a specific indication. For example, if you had a preclinical model with a mutation of some sort and you showed it really worked dramatically in one set, but not in the others. In the absence of either true synergy, not just an additive effect, or a specific population, I would not move drugs like this forward.

Dr. Alan Sandler: A negative phase II study is difficult issue for us as investigators and for the pharmaceutical companies because of the potential upside if there is a one out of hundred chance that maybe something happens. But if you were blinded and you just looked at the results from the phase II study to decide: (a) go ahead, (b) stop, you would stop.

Dr. Charles Butts: We have too many other agents and too few patients to go on trials to be wasting time with drugs that don’t seem to work.

Dr. Lynch: I think you do a phase II of that agent in the setting where you think it might work and you get the progression-free survival data. You have to have a stronger signal to make you feel going forward makes sense.

Dr. Philip Bonomi: Probably every one of these drugs has some possibility. You do the phase II single-agent study and then do a randomized phase II chemotherapy plus or minus or erlotinib plus or minus. If you don’t get a signal, then you have to back down.

Dr. Bruce Johnson: If this agent was being pursued only in lung cancer, it would likely die at this point. Here, though, there are two other tumors where it is likely active. Trying to figure out mechanistically if it can be adapted to lung cancer is a much different story than if this was being pursued solely as a lung cancer drug.

Dr. Herbst: This is a drug like others for which you have to identify the target, and lung cancer might not have this target in sufficient excess to allow for activity as a single agent. But we are moving towards understanding why tumors become resistant, what causes them to grow. Some day soon we are going to be using panels of gene arrays and protein assays on tumors and we will find that some tumors, maybe a small population, are resistant to an EGFR inhibitor because they are up-regulating one of these enzymes. We need to be characterizing these drugs and have them ready to go forward when we know more about the target. I suggest we need to go back to the tissue blocks for this lung trial and examine them for some of these enzymes to see if the levels correlate with activity.
References


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