A Phase I Study of Enzastaurin Combined with Pemetrexed in Advanced Non-small Cell Lung Cancer

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Introduction: Enzastaurin is an oral serine/threonine kinase inhibitor, which suppress signaling through protein kinase C-β and the phosphatidylinositol 3-kinase/AKT pathway. Preclinical studies suggested synergic antitumor activity of enzastaurin and pemetrexed. We conducted this phase I study to evaluate the safety, pharmacokinetics, and clinical activity of this combination in patients with previously treated advanced non-small cell lung cancer.

Methods: An oral daily dose of 500 mg enzastaurin was administered once daily (QD) or twice daily (BID) in combination with 500 mg/m^2 pemetrexed on day 1 in repeated 21-day cycles. Cycle 1 started with a 7-day enzastaurin lead-in treatment that preceded pemetrexed administration: a loading dose of 1125 mg enzastaurin on day 1 followed by a 500 mg total daily dose on days 2–7.

Results: Twelve patients were treated QD ($n = 6$) or BID ($n = 6$). One dose-limiting toxicity (grade 3 QTc prolongation) was reported in the QD cohort. Grade 3/4 hematological toxicities were slightly increased in the BID cohort compared with the QD cohort. After beginning the combination therapy, enzastaurin exposures decreased slightly but remained above the target plasma concentration of 1400 nmol/L. Compared with QD, there was a higher exposure with BID. The enzastaurin dosing regimen (QD or BID) had no effect on pemetrexed pharmacokinetics. Two patients had partial responses as defined by RECIST. Five patients received more than 10 cycles of treatment without disease progression.

Conclusions: Both schedules of enzastaurin in combination with pemetrexed were well tolerated and clinically active in patients with advanced non-small cell lung cancer.

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Lung cancer remains the leading cause of cancer mortality in the world. Platinum-based combination chemotherapy offers a survival benefit in patients with chemo-naïve advanced non-small cell lung cancer (NSCLC).[1,2] Nevertheless, the efficacy of platinum-based combination chemotherapy seems to have reached a therapeutic plateau, with a median survival of 8 to 12 months.[3,4] Several agents have been approved for second- and third-line therapy, including docetaxel,[5,6] pemetrexed[7], and erlotinib.[8] Nevertheless, they improve survival by only approximately 2.2 to 6.5 months compared with best supportive care.[5–8] A more effective therapy is needed.

Pemetrexed, a multitargeted antifolate, is currently approved as a first- and second-line therapy for locally advanced or metastatic NSCLC. The standard regimen of pemetrexed is 500 mg/m^2, administered intravenously (IV) on day 1 in repeated 21-day cycles, supplemented with folic acid and vitamin B_{12}.

Enzastaurin (LY317615), an oral serine/threonine kinase inhibitor, suppress signaling through protein kinase C (PKC)-β and the phosphatidylinositol 3-kinase/AKT pathway.[9–12] Enzastaurin is metabolized primarily by cytochrome P450 3A (CYP3A) to form a desmethylenepyrimidyl metabolite (LY326020) and a desmethyl metabolite (LY485912), two active metabolites with comparable potency against PKCβ. In vitro analysis has shown that the IC_{90} of enzastaurin for PKCβ is 70 nmol/L.[9] In light of the 95% plasma protein binding value of enzastaurin, the targeted mean steady state total concentration for clinical efficacy is estimated to be 1400 nmol/L. In a previous dose-escalation study (20–700 mg once daily [QD]) of patients with cancer, enzastaurin exposures reached a plateau above the targeted steady-state plasma concentration of 1400 nmol/L when administered at doses of 525 mg.[13] This dose was well tolerated, and enzastaurin at 500 mg QD demonstrated clinical activity as a single agent and in combination with cytotoxic agents.[14–17]

The combination of enzastaurin and pemetrexed has shown synergic antitumor activity in NSCLC cells.[18–20] Pre-

clinical studies revealed that enzastaurin suppressed thymidylate synthase (TS) expression through downregulation of E2F.[21] TS expression is one of the known sensitivity markers for pemetrexed, with pemetrexed treatment markedly increasing transcription of the *TS* gene. However, the combination of enzastaurin and pemetrexed significantly decrease TS activity and reduced glycogen synthase kinase-3beta/AKT phosphorylation and vascular endothelial growth factor secretion.[21] Therefore, evidence points to enzastaurin as a promising agent to increase the activity of pemetrexed.

In vitro analysis showed that twice daily (BID) dosing could maintain enzastaurin exposures above the targeted plasma concentration longer than QD dosing (data at Eli Lilly on file), which was confirmed in phase I studies.[15,17] We conducted this study to assess safety and tolerability of two dosing regimens of total daily 500 mg enzastaurin (QD or BID) in combination of pemetrexed.

The primary objective was to evaluate the safety of the combination of enzastaurin and pemetrexed in Japanese advanced NSCLC patients with prior systemic chemotherapy. The secondary objectives were to evaluate the toxicities of this combination and determine the pharmacokinetics of enzastaurin with or without pemetrexed. Antitumor activity of this combination was also assessed. We started with enzastaurin 500 mg QD in combination with pemetrexed to confirm safety in the QD cohorts. After this, we proceeded to 250 mg BID.

PATIENTS AND METHODS

Patients

Eligibility criteria included the following: histologically or cytologically documented NSCLC; clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy) or relapse after surgery; one or two prior systemic chemotherapy regimens, including at least one platinum-based regimen for NSCLC; presence of at least one measurable disease as defined by the Response Evaluation Criteria in Solid Tumors (RECIST); 20 to 75 years of age; Eastern Cooperative Oncology Group performance status of 0 to 2; adequate bone marrow reserve, hepatic, renal, and pulmonary function; predicted life expectancy of at least 12 weeks; recovery from toxicities of all previous therapies for NSCLC (had not received radiotherapy <28 days, chemotherapy <28 days, nitrosourea <42 days, hormone therapy <14 days, molecular targeted therapy <14 days, Uracil-Tegafur <14 days, or doxifluridine <14 days before enrollment).

Exclusion criteria were the following: interstitial pneumonia or pulmonary fibrosis detectable on radiologic evaluation; history of tube thoracostomy drainage for pleural effusion; inability to swallow capsules; myocardial infarction that occurred less than 6 months before entry; symptomatic angina pectoris, cardiac failure, arrhythmia not controlled by medication; prolonged QTc interval >450 milliseconds; symptomatic central nervous system metastasis; chronic use of nonsteroidal anti-inflammatory drugs; pregnancy; and serious comorbidity.

The study was approved by the institutional review boards of each participating institution and was conducted in accordance with the Declaration of Helsinki and good clinical practice and in compliance with all applicable laws and regulations. Written informed consent was given by each patient before study enrollment.

Study Design and Treatment Plan

This was an open-label, nonrandomized, multicenter study designed to assess safety and pharmacokinetics of enzastaurin administered QD (cohort 1) or BID (cohort 2) in combination with the standard dose of pemetrexed. During stage 1 of cycle 1, patients received a loading dose of enzastaurin (375 mg, 3 times, 1125 mg total dose) on day 1 followed by 500 mg total daily dose on days 2 through 7 of cycle 1 (Figure 1) to achieve the targeted steady-state concentration (1400 nmol/L).[9,13] During stage 2 of cycle 1, an oral daily dose of 500 mg enzastaurin was administered within half an hour after meals QD or BID. This was continued in cycle 2 and thereafter. Patients received standard pemetrexed as a 10-minute IV dose of 500 mg/m^2 on day 1 of stage 2 of cycle 1 and subsequently in a repeated 21-day cycle. On days of pemetrexed dosing, enzastaurin was administered after pemetrexed. We started with 500 mg QD (cohort 1), and after confirming safety, serially enrolled the BID cohorts and proceeded with 250 mg BID (cohort 2).

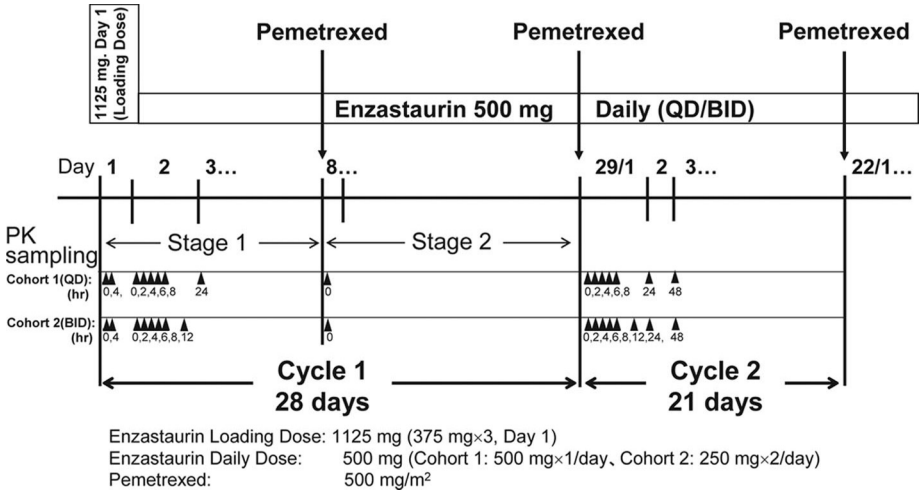

FIGURE 1. Study design and pharmacokinetic plan. An oral daily dose of 500 mg enzastaurin was given once daily (QD) in cohort 1 or twice daily (BID) in cohort 2 in combination with 500 mg/m^2 pemetrexed on day 1 in repeated 21-day cycles. Cycle 1 started with a 7-day enzastaurin lead-in treatment that preceded pemetrexed administration: a loading dose of 1125 mg enzastaurin on day 1 followed by 500 mg total daily dose on days 2–7. Pharmacokinetic (PK) sampling is indicated by chevrons. All patients received standard daily folate and vitamin B12 supplementation per standard treatment guidelines for pemetrexed infusion.

Each cohort was initially designed to enroll six patients. If three or more patients in each cohort experienced dose-limiting toxicities (DLTs) during cycle 1, the recruitment was to be ended. A DLT was defined as any of the following drug-related adverse events during cycle 1: (1) hematological toxicity, as determined by the Common Terminology Criteria for Adverse Events version 3.0: grade 4 neutropenia that persisted for 7 days or more, febrile neutropenia, grade 4 thrombocytopenia, or grade 3 thrombocytopenia with hemorrhage or requiring a blood transfusion; (2) grade 3/4 nonhematologic toxicities except for the following manageable events: nausea, vomiting, loss of appetite, fatigue, constipation, diarrhea, transient aspartate aminotransferase or alanine transaminase elevation, and transient electrolyte abnormality; (3) grade 3 corrected QT (QTc) prolongation >500 milliseconds or an increase ≥60 milliseconds over baseline QT measured at entry.

Safety Assessments

Physical examination results, vital signs (blood pressure, pulse rate, and body temperature), and performance status were evaluated at baseline, on day 1, and weekly during treatment. Complete blood count, serum chemistry, and urinalysis were performed at baseline and weekly during treatment. Twelve-lead electrocardiograms were recorded at baseline, 4–6 hours after the first dosing of enzastaurin on days 1 to 3 during stage 1, and at one point determined by the investigator between days 1 and 8 of each cycle. QTc values were obtained using Bazett's method of correction.[22]

Pharmacokinetic Measurements and Analyses

In cohort 1, blood samples for enzastaurin pharmacokinetics were collected on the following days (Figure 1): day 1 (before dosing and 4 hours after dosing), day 2 (before dosing and 2, 4, 6, 8, and 24 hours after dosing), and day 8 (before dosing) of cycle 1, and day 1 (before dosing and 2, 4, 6, 8, and 24 hours after dosing) of cycle 2. In cohort 2, blood samples were collected at the same points as cohort 1 except that the 24-hour collection time points after the first dose in cycles 1 and 2 was changed to 12 hours. Blood samples for pemetrexed pharmacokinetics were collected on day 1 (10 minutes, 2, 4, 6, 8, and 24 hours after dosing) and day 3 (before dosing) of cycle 2.

Pemetrexed plasma concentrations were measured using two validated high-pressure liquid chromatography with tandem mass spectrometry (LC/MS/MS) methods, high range and low range (SFBC Taylor, Princeton, NJ). Enzastaurin (LY317615) and its two active metabolites (LY326020 and LY485912) were also detected using a validated LC/MS/MS method (Advion BioSciences, Inc., Ithaca, NY).

Pharmacokinetic (PK) parameters for enzastaurin, its metabolites LY326020, LY485912, and pemetrexed were analyzed using noncompartmental methods (WinNonlin Enterprise Version 5.0.1; Pharsight Corporation, Mountain View, CA). PK parameters calculated for pemetrexed were area under the concentration versus time curve from time zero extrapolated to infinity (AUC_{0-inf}), maximum observed concentration (C_{max}), apparent clearance, apparent volume of distribution, and terminal half-life ($t_{[1/2]}$). PK parameters calculated were AUC_{0-24} for QD enzastaurin or AUC_{0-12} for BID enzastaurin, LY326020, LY485912, total analytes (enzastaurin + LY326020 + LY485912), C_{max}, and the observed time to reach peak drug concentration (t_{max}). PK parameters between cycle 1 and cycle 2 were compared to evaluate the effect of the loading dose to reach steady state in a short time. AUC_{0-24} for QD enzastaurin or AUC_{0-12} for BID enzastaurin, C_{max}, t_{max}, and metabolic ratio (metabolite AUC/parent AUC) were also calculated for LY326020 and LY485912.

Assessment of Tumor Response

Tumor measurement by radiologic imaging was done at baseline and every 42 days during treatment. Poststudy evaluation was conducted 30 ± 7 days after the last administration of enzastaurin. Tumor response was evaluated using the RECIST guideline.[23]

Statistical Analyses

All patients who received at least one enzastaurin dose were evaluated for safety, and those who received both enzastaurin and pemetrexed at least once were evaluated for efficacy. All analyses were descriptive, with no formal statistical test performed on the data from this study.

RESULTS

Patient Characteristics

Twelve patients were enrolled into the study at two cancer center hospitals in Japan from November 2007 to March 2008. All the 12 patients received at least one study treatment: six patients each were enrolled in the QD (cohort 1) and BID (cohort 2) groups. A single patient (cohort 1) discontinued the study before pemetrexed administration because of an adverse event. Baseline characteristics for the 12 patients are summarized in Table 1.

Dose Administration

In the study, a total of 91 cycles of therapy were completed, with a median number of cycles per patient of 4 (range, 1–19). Five patients (two in QD and three in BID) received more than 10 cycles of therapy (range, 13–19).

The reasons for discontinuation during the first four cycles were disease progression ($n = 5$) and adverse events ($n = 2$). Nine dosing delays of pemetrexed during the four cycles occurred in six patients because of adverse events (four in three patients), scheduling conflict (three in three patients), and others (two in one patient).

Toxicities

All 12 patients were evaluated for toxicities during the first four cycles. Table 2 lists all grade 3/4 drug-related toxicities; all drug-related toxicities with at least a 20% incidence in the overall population, regardless of grade, are also shown. One patient (BID) experienced grade 4 hematological toxicities: neutropenia, anemia, and thrombocytopenia. Grade 3 hematological toxicities occurred in four patients. Grade 4 nonhematological toxicities were not observed. All grade 3/4 toxicities were reversible and manageable, except for toxicities whose recovery could not be

TABLE 1. Patient Characteristics

	500 mg QD (n = 6)	250 mg BID (n = 6)	Total (n = 12)
Age, yr			
Median	63.5	59.0	61.5
Range	49–74	49–71	49–74
Gender, n (%)			
Male	5	3	8
Female	1	3	4
ECOG PS, n (%)			
0	4	4	8
1	2	2	4
Histology, n (%)			
Adenocarcinoma	5	3	8
Squamous cell carcinoma	1	2	3
Undifferentiated NSCLC	0	1	1
Disease stage, n (%)			
IIIB	1	3	4
IV	4	3	7
Relapse after surgery	1	0	1
Prior therapy, n (%)			
Chemotherapy[a]			
1 regimen	3	3	6
2 regimens	3	3	6
Surgery	1	0	1
Radiotherapy	*3*	*4*	7

[a] Cisplatin-gemcitabine, carboplatin-paclitaxel, cisplatin-S1, cisplatin-vinorelbine, docetaxel, gefitinib, and others.

ECOG, eastern cooperative oncology group; NSCLC, non-small cell lung cancer.

TABLE 2. All Grade 3/4 Toxicities and Toxicities with at Least 20% Incidence

	500 mg QD (n = 6)		250 mg BID (n = 6)		Overall (n = 6)	
Toxicity, n	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Nonhematological toxicity						
Chromaturia	5	0/0	6	0/0	11	0/0
Anorexia	5	0/0	5	0/0	10	0/0
Rash	5	0/0	5	0/0	10	0/0
Increased ALT	4	1/0	5	1/0	9	2/0
Increased AST	4	1/0	5	1/0	9	2/0
Fatigue	3	0/0	4	0/0	7	0/0
Nausea	4	0/0	3	0/0	7	0/0
Constipation	3	0/0	1	0/0	4	0/0
Discolored faeces	1	0/0	3	0/0	4	0/0
Hyponatremia	1	0/0	3	2/0	4	2/0
Increased LDH	1	0/0	3	0/0	4	0/0
Diarrhea	1	0/0	2	0/0	3	0/0
Fever	2	0/0	1	0/0	3	0/0
Hematuria	2	0/0	1	0/0	3	0/0
Hypoalbuminemia	1	0/0	2	0/0	3	0/0
Increased ALP	2	0/0	1	0/0	3	0/0
Arthralgia	2	1/0	0	0/0	2	1/0
QT prolongation	1	1/0	1	0/0	2	1/0
Hematological toxicity						
Anemia	5	0/0	5	0/1	10	0/1
Leukocytopenia	4	1/0	4	2/0	8	3/0
Lymphocytopenia	3	1/0	4	0/0	7	1/0
Neutropenia	3	1/0	3	2/1	6	3/1
Thrombocytopenia	2	0/0	2	1/1	4	1/1

Common Toxicity Criteria for Adverse Events version 3.0.

ALT, alanine transaminase; AST, aspartateaminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

confirmed because of disease progression. During the first four cycles, two patients discontinued the study because of drug-related toxicities: grade 3 QTc prolongation (QD) and grade 1 increased serum creatinine (BID). Four dosing delays of pemetrexed occurred in three patients (one in QD and two in BID) because of adverse events: neutropenia, thrombocytopenia, anemia, increased alanine transaminase, hyponatremia, and increased blood creatinine. Grade 3/4 hematological toxicities were slightly increased in BID dosing compared with QD dosing.

One DLT was reported: grade 3 QTc prolongation was observed 1 day after the enzastaurin loading dose in the QD cohort. This male patient with a history of coronary spastic angina experienced asymptomatic QTc prolongation ≥60 milliseconds over baseline (baseline: 430 milliseconds, post dose: 510 milliseconds). Enzastaurin was halted and his QTc normalized (420 milliseconds) in 6 days without the need of any medication. The patient was withdrawn from the study because of the event. There were no treatment-related deaths.

Treatment Response

Other than the single patient who discontinued before administration of pemetrexed because of DLT, all patients were assessed for response. Based on the results at the end of the study, two patients (one in QD and one in BID) (18%) achieved partial response (PR) and five patients (two in QD and three in BID) (45%) had stable disease (SD). Five patients (45%) received more than 10 cycles of treatment without disease progression.

Pharmacokinetics

PK parameters after 500 mg QD or 250 mg BID dosing of enzastaurin are shown in Table 3. The C_{max} for total analyte was reached within about 2 hours after BID dosing and about 5 hours after QD dosing. Total analytes in both BID and QD dosing declined in a monophasic manner after reaching maximum concentrations (Figure 2). Total analyte exposure (enzastaurin and its metabolites $AUC_{\tau,ss}$) of enzastaurin was approximately 21% and 3% lower, respectively, for 500 mg QD and 250 mg BID when administered with pemetrexed (cycle 2) compared with administration of enzastaurin alone (stage 1 in cycle 1). Maximum concentrations ($C_{max,ss}$) of total analyte were 14% and 2% lower, respectively, for 500 mg QD and 250 mg BID when administered with pemetrexed (cycle 2) compared with administration of enzastaurin alone (stage 1 in cycle 1) (Figure 2 and Table 3). Compared with QD dosing, there was a higher mean exposure for BID dosing. Total analyte concentrations reached

TABLE 3. Pharmacokinetic Parameters of Enzastaurin Total Analytes

	Geometric Mean (CV%)			
	500 mg QD		250 mg BID	
Parameter	Cycle 1, Day 2 One Day After Loading Dose[a]	Cycle 2, Day 1 (Steady State) + Pemetrexed	Cycle 1, Day 2 One Day After Loading Dose[a]	Cycle 2, Day 1 (Steady State) + Pemetrexed
N	6	4	6	5
C_{max} (nmol/L)	4870 (36.0)	4200 (50.1)	4420 (44.5)	4340 (31.3)
t_{max}[b] (h)	4.99 (0.00–6.00)	5.09 (2.02–8.02)	1.97 (1.78–3.95)	2.23 (1.93–4.22)
$AUC_{\tau,ss}$ (nmol/L·h)	86200 (36.1)	68500 (53.5)	42400 (49.3)	41100 (39.5)
$C_{av,ss}$ (nmol/L)	NC	2850 (53.5)	NC	3420 (39.5)

[a] Non-steady-statevalues, $AUC_{\tau,ss}$ = AUC(0–24 h) (QD) or AUC(0–12 h) (BID).
[b] Values are in median (range).
BID, twice daily; QD, once daily; *N*, numberof subjects used in the pharmacokinetic analysis; C_{max}, maximum plasma concentration; t_{max}, time to reach maximumconcentration; $AUC_{\tau,ss}$, area under the concentration versus timecurve during one dosing interval at steady state (QD = 24 h and BID = 12 h); $C_{av,ss}$, average drug concentration under steady-state conditions during multipledosing; CV, coefficient of variation; NC, not calculated.

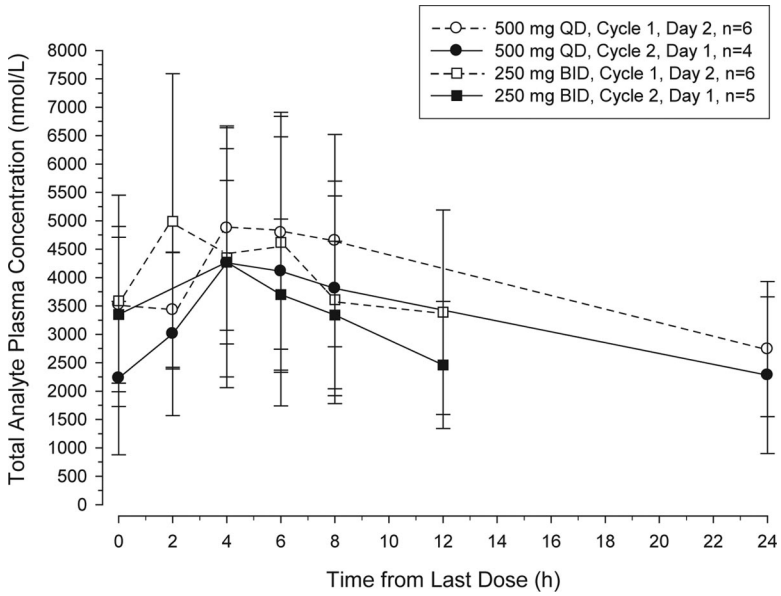

FIGURE 2. Plasma concentration of enzastaurin total analytes. Arithmetic mean (SD) total analytes (enzastaurin and its metabolites) plasma concentration time profiles after enzastaurin administered 500 mg once daily (QD) or 250 mg twice daily (BID) without concurrent pemetrexed (QD, open circle; BID, open square) or with concurrent pemetrexed (QD, black circle; BID, black square).

steady-state by day 8 of cycle 1, with mean steady-state concentrations (CV %) of 2850 nmol/L (53.5%) and 3420 nmol/L (39.5%) after QD and BID dosing, respectively (Figure 3). Total analyte exposure in cycle 1 was relatively higher than that in cycle 2, suggesting that the loading dose regimen of enzastaurin was instrumental in achieving a steady-state level of total analytes on day 2.

PK parameters of pemetrexed for QD and BID dosing are summarized in Table 4. Pemetrexed declined in a triphasic manner with an elimination half-life of 3.22 hours, which was consistent with previous observations of pemetrexed single dosing.[24] Interpatient variability in CL and Vss was less than 40%, implying constant systemic exposure to pemetrexed. Pemetrexed pharmacokinetics were not altered by enzastaurin dosing regimen, either QD or BID.

DISCUSSION

PKC overexpression and increased activity have been detected in a variety of tumors including several hematological malignancies, colon cancer, renal cell cancer, hepatocellular cancer, prostate cancer, and NSCLC.[25–30] Enzastaurin, a potent PKC inhibitor has been shown to have antiangiogenic and antitumor effects in NSCLC.[13,14,20,31] The synergic antitumor activity of enzastaurin and pemetrexed combination was shown in NSCLC cell lines,[18–20] and previous combination studies of enzastaurin with cytotoxic agents showed neither increased toxicity nor PK drug-drug interactions.[15,16,31,32] Therefore, in this study, we decided to assess safety of the recommended clinical doses of enzastaurin and pemetrexed (enzastaurin 500 mg/d and pemetrexed 500 mg/m^2).

The combination regimen was well tolerated for both QD and BID dosing. The observed range of grade 3/4 toxicities in this study was consistent with those seen in the monotherapy studies of enzastaurin and pemetrexed. All grade 3/4 toxicities, including DLT, were reversible and manageable. One patient with a history of ischemic heart disease developed grade 3 QTc prolongation that was considered a DLT. However, this event was asymptomatic and

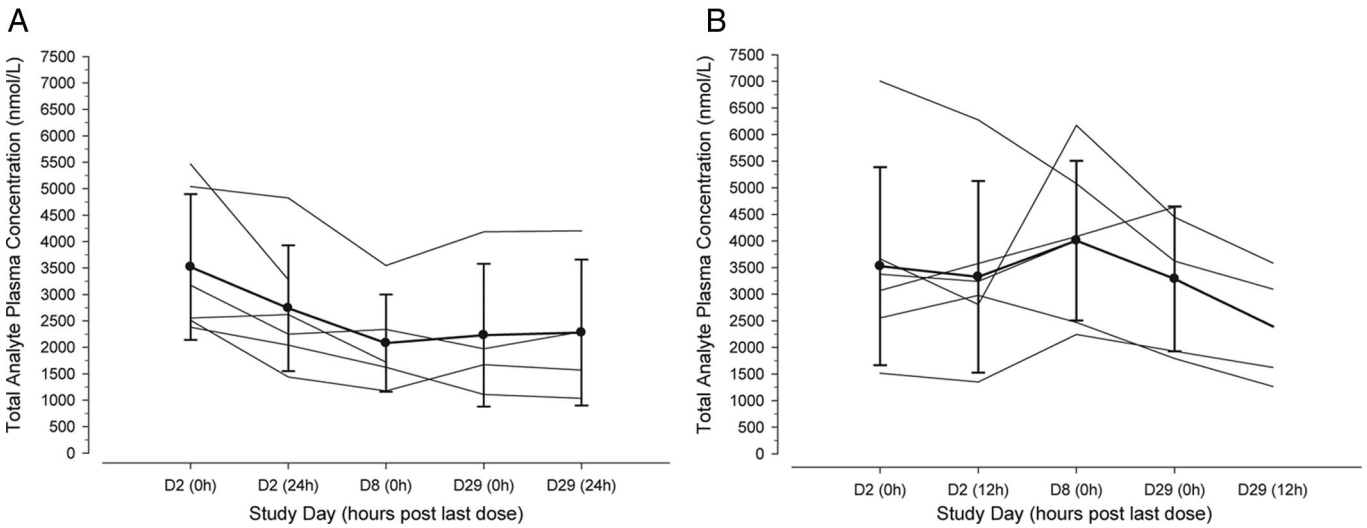

FIGURE 3. Pharmacokinetic result of enzastaurin: trough concentration of total analytes. Arithmetic mean (solid circles), SD, and individual trough concentrations (lines) of enzastaurin total analytes (enzastaurin and its metabolites) after enzastaurin administered 500 mg once daily (*A*) or 250 mg twice daily (*B*) without or with concurrent pemetrexed.

TABLE 4. Pemetrexed Pharmacokinetic Parameters After Enzastaurin Dose

	Geometric Mean (CV%)		
Parameter	**+Enzastaurin (Cycle 2) 500 mg QD**	**+Enzastaurin (Cycle 2) 250 mg BID**	**+Enzastaurin (Cycle 2) 250 mg BID and 500 mg QD**
N	4	5	9
C_{max} (μg/mL)	143 (20.4)	127 (10.6)	134 (15.8)
t_{max} [a] (h)	0.15 (0.15–0.18)	0.15 (0.15–0.17)	0.15 (0.15–0.18)
AUC(0–∞) (μg·h/mL)	265 (38.1)	236 (36.3)	248 (35.2)
CL (L/h/m^2)	1.88 (38.3)	2.12 (36.3)	2.01 (35.2)
V_{ss} (L/m^2)	5.70 (17.3)	6.12 (9.60)	5.93 (13.1)
$t_{1/2}$ [b] (h)	3.13 (2.67–3.60)	3.29 (2.42–4.85)	3.22 (2.42–4.85)

[a] Values are in median (range).
[b] Values are in geometric mean (range).
AUC(0–∞), area under the plasma concentration time curve; CL, systemic clearance; C_{max}, maximum plasma concentration; CV, coefficient of variation; *N*, number ofpatients; $t_{1/2}$, elimination half-life; t_{max}, time of maximal plasma concentration; V_{ss}, volume ofdistribution at steady state.

transient. In the enzastaurin preclinical toxicology study in dogs, prolonged QT and QTc values were observed after 5 weeks of dosing at a high daily dose of enzastaurin. In the enzastaurin phase I study in recurrent glioma patients, one grade 3 QTc prolongation was also reported.[33]

The PK results of this study indicated no significant PK interaction between enzastaurin and pemetrexed, which was consistent with a previously published phase I study report.[32] One possible reason for the absence of any effect on pharmacokinetics was the different pathways used for elimination. Pemetrexed is renally eliminated, whereas a phase I study using [^{14}C] enzastaurin indicated that enzastaurin undergoes extensive hepatic metabolism with minimal renal elimination (Eli Lilly and Company, Internal Clinical Study Report, October 2006). Based on these results, it is not likely that enzastaurin and its metabolites inhibit the renal elimination of pemetrexed. In fact, a previous combination study of enzastaurin with pemetrexed reported by Hanauske et al.[17] showed that pemetrexed pharmacokinetics (systemic clearance and half-life) did not seem to be altered by enzastaurin. In addition, it is not likely that pemetrexed inhibits the metabolism of enzastaurin by CYP3A4 because results from in vitro studies with human liver microsomes predicted that pemetrexed would not cause clinically significant inhibition of metabolic clearance of drugs metabolized by CYP3A, CYP2D6, CYP2C9, and CYP1A2.[34] In this study, the comparatively high concentrations observed in cycle 1 resulted from the large loading dose on day 1 of cycle 1. In addition, we confirmed two more findings that were reported by Hanauske et al.[17] First, the maximum concentrations ($C_{max,ss}$) of total analyte in both QD and BID regimens were slightly decreased in the presence of pemetrexed (14% QD and 2% BID in this study; 17% QD and 8% BID in the Hanauske phase Ib study). Second, the average steady-state plasma concentration ($C_{av,ss}$) of enzastaurin total analyte was slightly higher in the BID versus QD regimen (20% higher in this study and 11% higher in the Hanauske phase Ib study).

In light of the fact that two patients achieved PR, five patients achieved SD, and five patients remained on therapy for more than 9 months (13 cycles), the results from this study suggest that the combination of enzastaurin and pemetrexed might be beneficial in previously treated patients with advanced NSCLC. The histology of both the patients who achieved PR was nonsquamous cell carcinoma. One of three patients with squamous cell carcinoma remained on therapy for 14 cycles with SD, whereas PD was observed during cycle 1 for the other two patients. Further research is warranted to determine whether enzastaurin might improve the effect of pemetrexed that works preferentially in nonsquamous cell carcinoma.

In conclusion, combination therapy for enzastaurin administered QD or BID with pemetrexed was well tolerated and clinically active in patients with previously treated advanced NSCLC. Both dosing regimens of enzastaurin did not affect pemetrexed pharmacokinetics, and enzastaurin exposures remained above the targeted plasma concentration in the presence of pemetrexed. Enzastaurin exposures were higher with the BID regimen, with slightly more grade 3/4 hematological toxicities. These were manageable and BID dosing did not indicate any major tolerability issues.

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