A phase I safety, tolerability, and pharmacokinetic study of enzastaurin combined with capecitabine in patients with advanced solid tumors

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Enzastaurin, an oral inhibitor of protein kinase Cβ, affects signal transduction associated with angiogenesis, proliferation, and survival. Capecitabine is converted to 5-fluoruracil by thymidine phosphorylase, a putative angiogenic factor. The all-oral combination of the two drugs offers the potential for targeting angiogenesis in capecitabine-sensitive tumors with nonoverlapping toxicities. Patients with advanced cancer initially received single-agent enzastaurin to achieve steady-state concentrations (cycle 1). In subsequent 21-day cycles, enzastaurin was given orally, once daily, on days 1–21 and capecitabine orally, twice daily (b.i.d.), on days 1–14 in three dose-level cohorts. Three dose-escalation cohorts were studied: cohort 1 ($n=8$), 350 mg of enzastaurin + capecitabine (750 mg/m^2 b.i.d.); cohort 2 ($n=7$), enzastaurin (350 mg) + capecitabine (1000 mg/m^2 b.i.d.); cohort 3 ($n=12$), 525-mg capsules or 500-mg enzastaurin + capecitabine (1000 mg/m^2 b.i.d.). Further dose escalation was not pursued because of emerging data that enzastaurin systemic exposure did not increase at doses above 525 mg. Although a traditional toxicity-based maximum tolerated dose was not achieved, the highest dosing cohort represented a biologically relevant dose of enzastaurin, on the basis of preclinical data and correlative pharmacodynamic biomarker assays of protein kinase Cβ inhibition in peripheral blood mononucleocytes, in combination with a standard dose of capecitabine. For the 500/525-mg dose, ratios of total enzastaurin analyte geometric means (i.e. enzastaurin alone versus enzastaurin with capecitabine) reflected a trend toward decreased enzastaurin exposure, but did not reach statistical significance. The pharmacokinetic parameters of capecitabine with enzastaurin were similar to those previously reported for single-agent capecitabine. The regimen was well tolerated, without any consistent pattern of drug-related grade 3 or grade 4 toxicities being observed. Although no objective tumor responses were documented, five patients maintained stable disease for ≥ 6 months (range: 6–9.7 months). The recommended phase II dose of this combination, based on the results of this study, is enzastaurin at a daily dose of 500 mg (tablet formulation) and capecitabine (1000 mg/m^2, b.i.d.) on days 1–14 every 21 days. Further disease-directed studies are warranted, such as in malignancies in the treatment of which both capecitabine and inhibitors of angiogenesis have previously been benchmarked as being effective. *Anti-Cancer Drugs* 19:77–84 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Enzastaurin (LY317615) is an orally bioavailable, ATP-competitive, selective inhibitor of protein kinase Cβ (PKCβ), with an in-vitro IC_{50} in cell-free assays of 6 nmol/l [1]. Furthermore, its active metabolites, LY326020 and LY485912, are comparably potent as evidenced by their IC_{50} values against PKCβ *in vitro*. PKCβ-mediated signal transduction has been associated preclinically with the invasion [2], angiogenesis [3,4], and activation of prosurvival pathways [5]. Preclinically, enzastaurin has been noted to reduce proliferation and increase apoptosis in a range of human tumor cell lines in association with suppressed signal transduction through the phosphatidylinositol-3-kinase/AKT pathway [1]. Objective clinical responses with single-agent enzastaurin have been noted in high-grade gliomas [6] and prolonged disease stabilization in diffuse large B-cell lymphomas [7]. The most commonly reported toxicities in clinical trials of enzastaurin include fatigue, gastrointestinal disturbances, and reddish discoloration of the urine [8,9].

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Capecitabine is an orally bioavailable prodrug of 5-fluorouracil (5-FU) with proven efficacy in a range of different tumor types [10]. Conversion of capecitabine to 5-FU requires the enzyme thymidine phosphorylase (TP), a putative angiogenic factor that is also known as platelet-derived endothelial-cell growth factor, suggesting that malignancies sensitive to capecitabine can also be sensitive to antiangiogenic intervention [11]. The rationale for the combination of capecitabine and enzastaurin was based on their nonoverlapping toxicities, the convenience of an all-oral regimen, and the potential for enzastaurin to target angiogenesis in capecitabine-sensitive tumors. The goals of this phase I dose-escalation study were (i) to assess the safety and tolerability of coadministered enzastaurin and capecitabine; (ii) to investigate the effect of capecitabine on the pharmacokinetics (PKs) of enzastaurin and its metabolites; (iii) to explore the pharmacodynamic (PD) activity of enzastaurin on PKCβ in peripheral blood mononucleocytes at the plasma levels achieved; (iv) to determine the recommended phase II doses (RP2D) of the combination; and (v) to assess any preliminary evidence of the anticancer efficacy of the combination.

Patients and methods

Patient selection

Patients aged 18 years and above, with advanced, treatment-refractory solid malignancies and an Eastern Cooperative Oncology Group performance status ≤ 2, were considered eligible for this study. Standard inclusion criteria also included (i) no chemotherapy, radiotherapy, anticancer hormone therapy, or other investigational therapy for at least 4 weeks before study entry (2 weeks for palliative radiotherapy and 6 weeks for mitomycin C or nitrosoureas); (ii) adequate hematopoietic (absolute neutrophil count $\geq 1.5 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, and hemoglobin ≥ 9 g/dl), hepatic [bilirubin $\leq 1.5 \times$ upper limits of normal (ULN), alanine transaminase and aspartate transaminase $\leq 2.5 \times$ ULN, or $\leq 5 \times$ ULN, in the presence of known liver metastases], and renal (calculated creatinine clearance ≥ 50 ml/min using the Cockroft–Gault formula) function. Standard exclusion criteria included (i) untreated or symptomatic central nervous system metastases and (ii) QTc interval > 450 ms in men or > 470 ms in women. Female or male patients with childbearing potential would have to have this potential terminated or attenuated by surgery, menopause, or through the use of an approved contraceptive method during the study. Informed consent was obtained according to federal and institutional guidelines.

Study design and drug administration

This was a dual-site phase I dose-escalation study. Patients within individual dose cohorts were initially dosed to achieve steady-state concentration with enzastaurin alone (a 14- or 7-day cycle: cycle 1). The PK results of a single-agent phase I study of enzastaurin became available during the course of this study [8,9]. To allow patients to achieve near steady-state concentrations in less than 14 days, a change in this study was implemented by adding a loading dose on cycle 1 day 1 that consisted of a total dose of 1200 mg (three 400-mg tablets, 5–6 h apart) and a single daily dose at the dosage used within cycle 2, but with a reduced lead-in period of cycle 1 from 14 to 7 days. The 7-day lead-in period was administered to one patient in each of the cohorts 1 and 2 and all 12 patients within cohort 3; all the others received the 14-day lead-in dosing (see Results for the cohort dose-level details). From cycle 2 onwards, enzastaurin was administered continuously in 21-day cycles, whereas capecitabine was administered twice daily (b.i.d.) for 14 days every 21 days. Enzastaurin doses in the continuous-dosing phase ranged from 350 to 525 mg/day, whereas the capecitabine doses ranged from 750 to 1000 mg/m^2 b.i.d. Enzastaurin and capecitabine were administered orally within 30 min of eating breakfast (and in the fed state during the b.i.d. dosing of capecitabine and the thrice daily loading phase of enzastaurin).

Toxicities were graded according to the National Cancer Institute's Common Toxicity Criteria (CTC) version 2. Each cohort was initially designed to enroll three patients, with cohort recruitment at the next highest dose level being permitted once the first three patients at a given dose level had completed cycles 1 and 2 without experiencing dose-limiting toxicities (DLTs). DLTs were defined as any nonhematologic toxicity $\geq$ grade 3, or hematologic toxicity $\geq$ grade 4, or any toxicity that resulted in a dose delay of more than 14 days. If even one out of the three patients experienced a DLT, then the cohort would have to be expanded to include a minimum of six patients.

If two patients in a cohort experienced DLTs, then the maximum tolerated dose was deemed to have been exceeded. Taking into account all available data, the RP2D could be set as the maximum tolerated dose (the dose level at which one in six patients experienced a DLT) or lower. During the course of the study, however, the results of a single-agent phase I study of enzastaurin became available [9]. As doses above 525 mg daily did not appear to produce significant increases in systemic exposure to enzastaurin and its metabolites, it was determined that if dose level 3 was reached without significant toxicity, this would be set as the RP2D. Therapy continued in individual patients until disease progression or unacceptable toxicity occurred. In the event of DLTs, dosing in that particular patient could be held for up to 2 weeks; and if the toxicities had resolved to CTC grade 1 or less, or to the patient's baseline grading, then capecitabine could be reintroduced after a 25% dose reduction or at the next lowest dose level. Dose reductions of enzastaurin were not permitted. Intrapatient dose escalation was not permitted.

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Enzastaurin was supplied either as 100- and 25-mg capsules or as 100- and 200-mg tablets (Eli Lilly, Indianapolis, Indiana, USA). During the course of the study, the results of a relative bioavailability study in healthy volunteers suggested that, in the fed state, capsule and tablet formulations of enzastaurin produced comparable exposures (data at Eli Lilly on file). As the tablet was the preferred dosing form, the formulation of enzastaurin was changed from capsule to tablet in a protocol amendment that affected cohort 3. Two cohort 3 patients only received the 500-mg tablets, whereas 10 patients had begun daily dosing with 525-mg capsules and had converted to 500-mg tablets at the commencement of their next cycle after the approval of the amendment. Of note, however, no formulation changes occurred for any individual during the PK analysis phase. Over the course of the study, a worldwide enzastaurin supply shortage led to a hold in accrual within this study for approximately 1 year. Capecitabine was supplied as tablets of 150 or 500 mg (Hoffman-La Roche, Basel, Switzerland) and dosed according to the body surface area.

Pretreatment and follow-up studies

Medical histories and examinations were performed at baseline and on day 1 of each cycle. Routine laboratory safety/toxicity studies (complete blood counts and comprehensive metabolic profiles) were performed at baseline, weekly during cycle 2, and on day 1 of each cycle thereafter. Twelve-lead ECGs were performed at baseline, before, and 4 h after drug administration on cycle 2, day 1, and immediately before dosing on cycle 3, day 1. Radiological tumor assessments and evaluations according to Response Evaluation Criteria in Solid Tumors guidelines were performed within the 4 weeks before the commencement of the study, before cycle 4, and after every two subsequent cycles thereafter.

Blood sampling and drug assay

Blood sampling for PK characterization was originally planned only for those patients treated at the RP2D; however, a protocol amendment later expanded the sampling to include patients at all dose levels. Sampling only occurred in individuals receiving the thrice daily loading dose of enzastaurin in cycle 1. Blood sampling for the quantitation of enzastaurin and its metabolites (LY326020 and LY485912) occurred on cycle 1, days 1–3, before dosing; on cycle 1, day 7, before dosing and at 2, 4, 6, 8, and 24 h after dosing; and on cycle 2, day 8, before dosing and at 0.5, 1, 2, 4, 6, 8, and 24 h after dosing. Blood sampling for the quantitation of capecitabine and its metabolites [5′-deoxy-5-fluorouridine (5′-DFUR) and 5-FU] occurred on cycle 2, day 1 before dosing and, on cycle 2, day 8, before dosing and at 0.5, 1, 2, 4, 6, 8, and 12 h after dosing. Plasma samples were analyzed for enzastaurin, capecitabine, and their metabolites using validated liquid chromatography–tandem mass spectrometry methods (Advion BioSciences, Ithaca, New York, USA) [9].

Pharmacokinetic analysis

The PK parameters for enzastaurin, LY326020, and LY485912, and for capecitabine, 5′-DFUR, and 5-FU were analyzed by standard noncompartmental methods using WinNonlin Version 4.1. (Pharsight, Mountain View, California, USA). The primary PK parameters for enzastaurin were the observed time to reach peak drug concentrations at steady state ($t_{max,ss}$), maximum observed concentration at steady state ($C_{max,ss}$), area under the plasma concentration versus time curve for the dosing interval at steady state ($AUC_{\tau,ss}$), the mean drug concentration during the dosing interval ($C_{av,ss}$), and apparent plasma clearance (CL/F). The same parameters (with the exception of CL/F) were reported for LY326020 and LY485912 and for total analytes (enzastaurin + LY326020 + LY485912). The metabolic ratio (metabolite $AUC_{\tau,ss}$/parent $AUC_{\tau,ss}$) was also calculated for each metabolite.

PK parameters calculated for capecitabine and its metabolites were $t_{max,ss}$, $C_{max,ss}$, and $AUC(0–t_{last})$, where t was the time of the last quantifiable concentration.

$AUC_{\tau,ss}$, $C_{max,ss}$, CL/F, and metabolic ratios were compared between cycles 1 and 2 to evaluate the effect of capecitabine on the PK of enzastaurin, LY326020, LY485912, and total analytes. Log-transformed data were analyzed using a mixed-effect model, with treatment as a fixed effect and patient as a random effect, to determine geometric means, geometric mean ratios, and their 90% confidence intervals. Any effect of capecitabine on t_{max} was evaluated using the Wilcoxon signed-rank test.

Pharmacodynamic sampling and phosphorylated protein kinase C substrate-detection assay

Venous blood for the preparation of peripheral blood mononucleocytes (PBMCs) was collected from 17 patients into Becton Dickson Vacutainer CPT tubes containing sodium heparin anticoagulant (Becton Dickinson, Franklin Lakes, New Jersey, USA) before dosing on cycle 1, day 1 and 1–4 h after dosing on cycle 1, day 1 and on cycle 2, day 14. The full details of the PBMC preparation and phosphorylated PKC substrate-detection assay used have been reported elsewhere [12]. Samples were analyzed from the nine patients whose samples had been received and processed in a timely manner (< 29 h after sampling) and in whom a complete data set was available. Eight of these nine patients had received enzastaurin 525-mg capsules and one had received 350-mg capsules during the sampling period.

Results

A total of 27 patients were enrolled into this study between November 2002 and July 2005. Patient characteristics are depicted in Table 1, and the courses

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administered as a function of dose level are depicted in Table 2.

A total of 124 cycles of therapy were completed (mean/patient = 4.6; range: 2–14). Dose reductions of capecitabine were allowed but none occurred. Omissions of 54 capecitabine doses took place, 19 of which were considered to be capecitabine related and 11 of which occurred in the same individual for intermittent emesis. No dose reductions were allowed for enzastaurin; nevertheless, 43 dose omissions occurred, 18 of which were considered to be enzastaurin-related and 16 of which occurred in the same individual for intermittent emesis.

Toxicities

No grade 4 drug-related toxicities occurred. Table 3 lists the study drug-related toxicities by cohort and CTC grade. Study-drug-related toxicities occurring in more than 20% of the patients were nausea (48%), fatigue (33%), vomiting (26%), and diarrhea (26%). Reddish discoloration of the urine, palmar/plantar dysesthesia, and skin/nail changes all occurred in five of the 27 patients (19%). Four patients experienced grade 1 QTc prolongation (> 480 ms and asymptomatic) during the course of the study.

Two DLTs were observed in cohort 2. In one case, a female patient, with a history of arrythmias and mitral valve prolapse experienced asymptomatic (grade 1) QTc prolongation on C2D1 (baseline = 452 ms; predose = 473 ms; 4 h postdose = 494 ms). Both capecitabine and enzastaurin were held and the patient was hospitalized for cardiac investigations and magnesium repletion until QTc normalization 3 days later (peak QTc = 508 ms). The patient was subsequently withdrawn from the study without rechallenge. At this point, additional patients were enrolled into cohort 2. The last patient of the seven enrolled in this cohort experienced a grade 3 coronary vasospasm on cycle 2, day 3, which was attributed to capecitabine, and IRB approval was obtained to continue on enzastaurin alone. For cohort 2 as a whole, there was one DLT associated with enzastaurin (QTc prolongation) and one DLT that seemed to be specifically associated with capecitabine (vasospasm) in seven patients. The protocol was amended to count the DLTs that were considered to be specific to capecitabine separately, thus allowing dose escalation to cohort 3. Although the amendment was under review, cohort 1 was expanded up to a total of eight patients, none of whom experienced DLTs. Subsequent to approval of the amendment, cohort 3 then enrolled 12 patients, with only one patient experiencing a DLT. This patient had advanced gastric cancer and had both drugs held at the end of cycle 2 owing to grade 3 diarrhea and fatigue. Eight days later, a paracentesis was performed, which was followed 2 days later by abdominal pain and hypotension, and by death 3 days later due to bowel perforation. No bowel perforations had previously been reported with either capecitabine or enzastaurin; although, owing to the temporal relationship, the possibility of the event being drug-related could not be ruled out. One patient in cohort 1 also died during the

Table 1 Patient demographics (*N* = 27)

	No. of patients
Men/women	13/14
Mean age	56.7 years (range: 34–75)
ECOG performance status	
0	13
1	14
Primary site of malignant diagnosis	
Breast	5
Lung/bronchial	2
Pancreas	3
Liver/hepatocellular	2
Kidney	3
Colorectal	4
Other (adenoid cystic carcinoma of salivary gland, esophagus, stomach, carcinoma NOS, adenocarcinoma NOS, chondrosarcoma, sarcoma, hemangiopericytoma)	1 each
Prior anticancer therapies	
Chemotherapy	24
Radiotherapy	13
Hormonal therapy	4
Immunotherapy	3

ECOG, Eastern Cooperative Oncology Scale; NOS, not otherwise specified.

Table 2 Dose-escalation scheme

	Enzastaurin (mg/day, days 1–21)	Capecitabine (mg/m^2 twice daily, days 1–14)	No. of patients	DLTs[d]
Cohort 1	350 (capsules)	750	8[a]	None
Cohort 2	350 (capsules)	1000	7[b]	QTc prolongation[e], Chest pain[f]
Cohort 3	525 (capsules) or 500 (tablets)	1000	12[c]	Diarrhea[g], fatigue[g]

[a]Five additional patients were enrolled in dose level 1 as per the investigator's decision after a DLT (QTc prolongation) occurred in dose level 2.
[b]Three additional patients were enrolled in dose level 2 after one DLT was observed; another patient was enrolled as a replacement to one of the additional three patients who experienced a capecitabine-related DLT (chest pain).
[c]Dose level 3 was expanded up to 12 patients following identification as the recommended phase II dose.
[d]DLT assessment period was cycle 1 and 2.
[e]Asymptomatic QTc prolongation (up to 508 ms) occurred in a patient with a history of arrythmias and mitral valve prolapse, who was hospitalized for magnesium repletion.
[f]Chest pain attributed to capecitabine-induced grade 3 coronary vasospasm (capecitabine-induced toxicities counted separately in dose-escalation decision making following protocol amendment).
[g]Diarrhea and fatigue occurred in same patient at the end of cycle 2, before a paracentesis (8 days later) and death from bowel perforation 13 days later.
DLT, dose-limiting toxicity.

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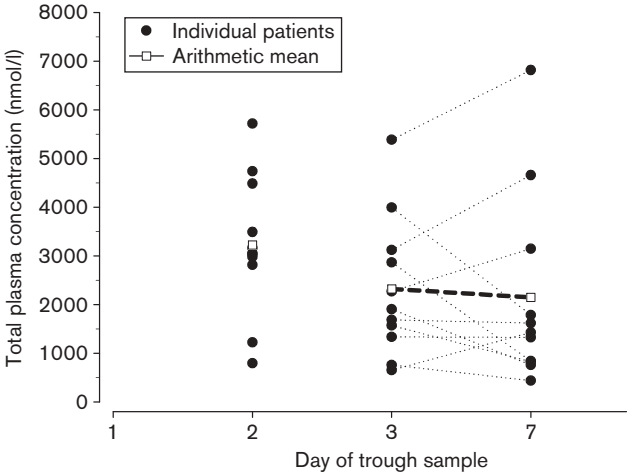
Table 3 Study drug-related adverse events summary[a,b,c]

	Cohort 1 (*n*)	Cohort 2 (*n*)	Cohort 3 (*n*)
Grade 3 toxicities by cohort (all patients):			
Fatigue			1
Palmar/plantar dysethesia		1	1
Chest pain		1	
Bowel perforation			1
Anemia			1
Vomiting			1
Headache	1		
Diarrhea			1
Grade 1/2 toxicities by cohort [all patients (grade 2); ≥ 3 patients (grade 1)]			
Fatigue	2	2	4
Skin/nail changes	2		3
Headache		1	
Palmar/plantar dysethesia		1	1
Dyspepsia		1	
Nausea	3	3	7
Vomiting	1	1	4
Dry eyes		1	
Dyspnea			2
Anorexia/weight loss			1
Abdominal pain/bloating			2
Loose stool/diarrhea	2	1	4
Flatulence			1
Arthralgia/mylagia			1
Reddish urine discoloration	1		4
QTc prolongation		1	3

[a]Patients who had the same event in subsequent cycles only counted once.
[b]Only maximum CTC grade counted.
[c]No grade 4 drug-related toxicities were observed.
CTC, Common Toxicity Criteria.

Fig. 1



Predose concentrations of enzastaurin total analytes on cycle 1, days 2, 3, and 7 following a loading dose on cycle 1, day 1 (cohort 3, $N=11$).

study. The patient had breast cancer metastatic to the liver, lung, and bone; and had both study drugs held at the end of cycle 2 owing to hyperbilirubinemia and hypercalcemia, neither of which was considered to be drug related. The patient was too ill for further imaging and died 17 days later owing to progressive breast cancer.

Pharmacokinetics

PK analysis for enzastaurin and its metabolites was performed in 14 patients; however, one patient was < 75% adherent to taking the study medication owing to intermittent emesis, and was excluded from the subsequent PK analysis. All of these 13 patients (six men and seven women) received the loading dose on cycle 1, day 1. Data on total analyte concentrations during cycle 1 for 11 patients within cohort 3 (two patients on tablets and nine on capsules) are shown in Fig. 1. Owing to the shorter interdose interval (approximately 10 h) from the last loading dose on day 1 to predose on day 2, compared with the 24-h dosing interval thereafter, the higher arithmetic mean predose concentrations on cycle 1, day 2, compared with those on cycle 1, day 3 and on cycle 1, day 7, were expected. Comparisons between cycle 1, day 3 and cycle 1, day 7 indicate similar ranges and mean exposures, supporting the attainment of steady-state concentrations following the day 1 loading dose and the daily dosing up to the cycle 1, day 7 regimen.

To address any effect of capecitabine on enzastaurin exposure, enzastaurin PK data from both cycles 1 and 2 were available from five patients receiving the 525-mg dose and one patient receiving the 500-mg dose. Total analyte arithmetic means (± standard errors) are shown graphically in Fig. 2. Derived PK parameters of enzastaurin and its metabolites (geometric means) from cycles 1 and 2 are shown in Table 4. Comparative PK analyses of t_{max} using the Wilcoxon signed-rank test did not yield any statistically significant information (data not shown).

PK data were available for capecitabine and its metabolites in 10 patients (six men and four women) from cycle 2. One patient received 750 mg/m^2 b.i.d. of capecitabine, and nine received 1000 mg/m^2 b.i.d. of it on days 1–14. As the doses were restricted by the available size of capecitabine tablets, the actual mg/m^2 dose varied slightly between patients; therefore, all concentrations were normalized to the standard single agent 1250-mg/m^2 b.i.d. dose before the analysis. Summary PK data of capecitabine, 5′-DFUR and 5-FU are shown in Table 5.

Pharmacodynamics

Treatment with enzastaurin produced a statistically significant decrease in PBMC PKC activity compared with that at baseline, as measured using a multiparameter flow cytometry assay described elsewhere [12].

Antitumor activity

Ten patients had a best response of stable disease during the study. Of these 10 patients, five remained on the study for 6 months or longer (Table 6). The details of their tumor growth dynamics before the start of the study were not recorded.

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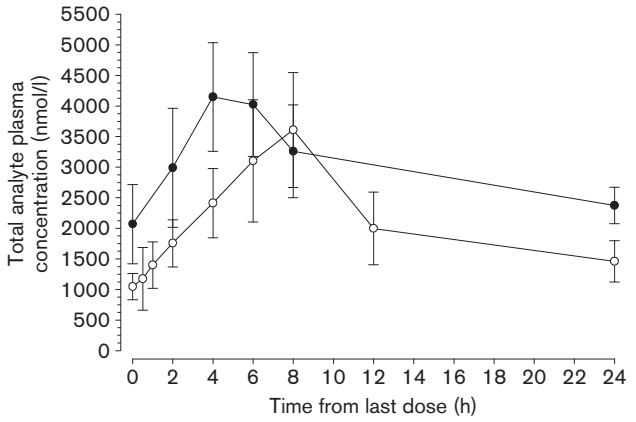
Discussion

Enzastaurin is a small molecule inhibitor of PKC, which affects signal transduction associated with angiogenesis, proliferation, and survival. Capecitabine is converted to 5-FU by TP, a putative angiogenic factor. The combination of enzastaurin and capecitabine was pursued on the basis of the anticipated nonoverlapping toxicities of the two agents, the potential for mechanistic additivity or synergy against tumors with a predominant angiogenic phenotype, and the convenience of an all-oral regimen. This phase I combination study assessed the tolerability of the combination, the potential for PK interaction, the achievement of PD-relevant enzastaurin exposures, and preliminary evidence of anticancer activity.

Fig. 2



Total analyte arithmetic means (± standard error) plasma concentration–time profiles in patients receiving multiple daily doses of enzastaurin with and without coadministration of capecitabine.

The combination was well tolerated across a range of doses with no grade 4 toxicities being observed. Although two patients with grade 3 palmar–plantar dysesthesia were noted, no other consistent pattern of grade 3 toxicities attributable to either study drug was detectable (Table 3). A certain complexity is inherent in combination studies in correctly ascribing toxicities to the combination itself or to each drug during decisions regarding dose escalation, given the expected side effects of the individual drugs. A protocol amendment was introduced during the study to tabulate those toxicities clearly associated with capecitabine separately from those of enzastaurin, in the assessment of DLTs. The counter argument, that a PD or PK interaction could exaggerate any drug-specific effects of either drug within the combination is, however, noted. A single case of grade 1 QTc prolongation, which was potentially attributable to the study drugs, was observed and it caused the withdrawal from the study during the DLT assessment period at 350-mg enzastaurin (cohort 2). The three other

Table 4 Enzastaurin, LY326020, LY485912, and total analyte pharmacokinetic parameters in patients administered enzastaurin daily and sampled during both cycle 1 (enzastaurin) and cycle 2 (enzastaurin with capecitabine)

	Geometric mean (CV%)							
	Enzastaurin		LY326020		LY485912		Total analyte	
	Cycle 1 $N=6$	Cycle 2 $N=6$	Cycle 1 $N=6$	Cycle 2 $N=6$	Cycle 1 $N=6$	Cycle 2 $N=6$	Cycle 1 $N=6$	Cycle 2 $N=6$
$C_{max,ss}$ (nmol/l)	2480 (69.9)	1650 (130)	893 (51.8)	721 (80.1)	365 (77.6)	220 (126)	3800 (61.9)	2520 (114)
E + C/E ratio[d]	0.66		0.81		0.6		0.66	
(90%CI; *P* value)	(0.4–1.11; 0.17)		(0.56–1.17; 0.3)		(0.35–1.02; 0.11)		(0.42–1.06; 0.14)	
t_{max}[a] (h)	5.21 (2.00–6.00)	4.96 (1.00–6.08)	6 (4.00–7.50)	7.59 (2.03–8.08)	6 (4.42–8.00)	7.59 (2.03–8.08)	5.21 (2.00–6.00)	4.96 (2.00–6.08)
$AUC_{\tau,ss}$ (nmol/l h)	44100[b] (45.8)	27000[c] (43.5)	23000[b] (57)	18500[c] (47.8)	9360[b] (58.5)	5430[c] (15.5)	80600[b] (33.2)	51100[c] (42)
E + C/E ratio[d]	0.61		0.89		0.58		0.65	
(90%CI; *P* value)	(0.36–1.05; 0.13)		(0.75–1.05; 0.2)		(0.36–0.93; 0.07)		(0.38–1.1; 0.15)	
$C_{av,ss}$ (nmol/l)	1840[b] – 45.8	1130[c] – 43.5	958[b] – 57	769[c] – 47.8	390[b] – 58.4	226[c] – 15.5	3360[b] – 33.2	2130[c] – 42
CL/F (l/h)	22.8[b](48.5)	37.3[c](43.2)						
			NC	NC	NC	NC	NC	NC
E + C/E ratio[d]	1.64		NC		NC		NC	
(90%CI; *P* value)	(0.94–2.84; 0.13)							
MR			0.521[b](92.6)	0.684[c](7.92)	0.212[b](21.2)	0.201[c](27)		
	NC	NC					NC	NC

$AUC_{\tau,ss}$, area under the concentration vs. time curve during one dosing interval at steady-state; $C_{av,ss}$, the predicted average drug concentration under steady-state conditions during multiple dosing; CI, confidence interval; CL/F, apparent total body clearance calculated after oral dosing; $C_{max,ss}$, maximum plasma concentration at steady state; CV, coefficient of variation; E + C/E, enzastaurin + capecitabine/enzastaurin alone; MR, metabolic ratio; *N*, number of patients; NC, not calculated; t_{max}, median time to reach maximum concentration.

[a]Median (range).

[b]$N_{pk}=4$.

[c]$N_{pk}=5$.

[d]Ratio (enzastaurin + capecitabine vs. enzastaurin alone).

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Table 5 Pharmacokinetic parameters of capecitabine, and its metabolites 5′-DFUR and 5-FU, when administered in combination with enzastaurin

	Geometric mean (CV%)		
	Capecitabine ($n=10$)	5′-DFUR ($n=10$)	5-FU ($n=10$)
$C_{max,ss}$ (μg/ml)	2.6	3.37	0.171
	152	106	115
t_{max}[a] (h)	1.76	1.96	1.96
	0.42–7.08	0.92–7.08	0.92–7.08
$AUC_{0-tlast}$ (μg h/ml)	3.05	4.94	0.263
	79.5	51.6	81.9

AUC, area under the curve; $C_{max,ss}$, maximum plasma concentration at steady state; CV, coefficient of variation; t_{max}, median time to reach maximum concentration; 5′-DFUR, 5-fluorouridine; 5-FU, 5-fluorouracil.
[a]Median (range).

Table 6 Summary of patients on study for ≥ 6 months

Tumor type	Cohort	Months on-study	Prior treatments[a]	Prior capecitabine
Breast	3	9.7	6 systemic therapies	No
Adenoid cystic carcinoma of salivary gland	3	9.0	0 systemic therapies, 1 radiotherapy	No
Pancreas	2	7.2	2 systemic therapies	No
Hemangiopericytoma	3	6.5	4 systemic therapies, 1 radiotherapy	Yes
NSCLC	1	6.0	3 systemic therapies	No

Systemic therapies = chemotherapy + biological therapy.
NSCLC, nonsmall cell lung cancer.
[a]Regimens for metastatic disease.

cases of grade 1 QTc prolongation occurred in cohort 3. QTc prolongation was also observed at the highest doses (525 and 700 mg) explored in the dose-escalation study of enzastaurin as a single agent [9]. No patient in either study, however, suffered any symptoms from these QTc effects; whether such isolated ECG changes have any true clinical significance is unclear.

The safety and tolerability data from the highest dose level in this study included patients receiving enzastaurin as either 500-mg tablets or 525-mg capsules. As data from a companion single-agent dose-escalation study of enzastaurin suggested that enzastaurin exposures plateaued above a dose of 525 mg/day, dose escalation of enzastaurin beyond 500/525 mg was not undertaken within this study [8,9]: the RP2D of enzastaurin in combination with capecitabine was 500 mg of enzastaurin daily (tablet formulation) and 1000 mg/m^2 of capecitabine b.i.d. on days 1–14 in a 21-day cycle.

Given the caveat of the relatively small numbers of patients available for paired PK analysis (enzastaurin alone and enzastaurin in the presence of capecitabine), capecitabine did not affect enzastaurin total analyte exposures in a statistically significant manner. A nonstatistically significant trend was, however, seen toward lower total analyte exposures (lower C_{max}, lower AUC, and longer time to t_{max}) in the presence of capecitabine, potentially indicating an effect on the absorption and/or clearance of enzastaurin by capecitabine (Fig. 2). In addition, a nonstatistically significant trend, based on the derived PK parameters, toward an apparently greater enzastaurin clearance in the presence of capecitabine was observed (Table 4). Although the formulation of the enzastaurin was changed from capsules to tablets during the study, this change did not occur during the PK sampling period. One patient within the PK sampling period received only the 525-mg capsules rather than the 500-mg tablets, which might have potentially altered the absolute figures; however, it should not have influenced the comparative data with and without capecitabine. Previous studies of enzastaurin with single-agent healthy volunteers (5–400 mg/day) and previous cancer patients (20–700 mg/day) have all shown high intraparticipant and interparticipant PK variability (data at Eli Lilly on file; [8,9]). High intraparticipant and interparticipant variability in terms of dose-for-dose total analyte trough levels is also apparent within this study (Fig. 1). Owing to this high background PK variability, only a very large interaction on the scale of, for example, capecitabine's interaction with warfarin is likely to be clinically significant [13]. Little chance exists that such a large interaction would have been missed by this study.

The study was not designed to detect an effect of enzastaurin on capecitabine exposure. PK parameters of capecitabine, 5′-DFUR, and 5-FU have been reported in the literature following single and multiple dosing [14–16]. The published ranges of C_{max} (2.68–5.05 mg/l), t_{max} (1.5–2.0 h), and AUC (5.62–6.46 mg h/l) values are broadly in line with the results that we obtained for capecitabine in this study. Similarly, our results for 5′-DFUR and 5-FU were also broadly within the published PK ranges (data not shown).

The enzastaurin plasma levels achieved in cohort 3 were all reassuringly within the presumed biologically effective range. In-vitro experiments have shown 90% (IC_{90}) inhibition of PKCβ enzymatic activity at 70 nmol/l (data at Eli Lilly on file). As preliminary data suggest that enzastaurin is approximately 95% protein-bound in human plasma, to achieve free concentrations of above 70 nmol/l, total plasma concentrations of above 1400 nmol/l would be required. Owing to missing data values, the total analyte $C_{av,ss}$ was calculated for only four patients in cycle 1 and five patients in cycle 2 (Table 4). In each of the four patients, however, in whom data were available for both cycles, the total analyte $C_{av,ss}$ values were all above 1400 nmol/l ($\geq$ 1780 nmol/l) when administered either with or without capecitabine at doses of 525 mg ($n = 3$) or 500 mg ($n = 1$). In addition, a PD assay developed as part of this study to show PKCβ inhibition within PBMCs demonstrated biologic activity at the

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RP2D (eight of the nine PD samples analyzed were from patients receiving 525 mg of enzastaurin) [12]. At 1 umol/l *in vitro*, the IC_{90}s of PKCγ, PKCδ, PKCθ, PKCε, and PKCξ, in addition to PKCβ, are all exceeded. This raises the possibility that, at clinically achievable plasma levels in the presence of capecitabine, there is the potential for relevant activity on a number of other PKC isoforms [1,17,18]. No objective responses were seen. It is possible that the PK exposures and PD relating to target drug sensitivity and duration of effect might vary significantly between PBMCs and malignant cells that are contained within a tumor mass; nevertheless, the lack of clinical responses is more likely to reflect a lack of relevant molecular pathway addiction in this heavily pretreated population.

In conclusion, the RP2D of enzastaurin in combination with capecitabine, based on good tolerability and safety, is 500 mg of enzastaurin daily (tablet formulation) and 1000 mg/m^2 of capecitabine b.i.d. on days 1–14 in a 21-day cycle. Future studies of the combination should consider targeting the regimen in those tumor types considered likely to respond to capecitabine. They should also aim to assess TP expression in available tumor tissue to further explore the hypothesis that, in addition to acting as a marker for capecitabine sensitivity [19], TP might also act as a marker for effective antiangiogenic intervention with drugs such as enzastaurin.

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